ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editorial Director

Why Bother with Chemical Methods?

We have noted more than once recently indications that what once was taken as a facetious remark is now tinged with seriousness. Jet- and atomic-age analysts using instruments costing in the range of $10,000 to $100,000 actually are beginning to believe and say that anyone who uses chemical wet methods of analysis is either stupid or cannot afford to be up to date.

Part of this attitude undoubtedly can be traced to the fact that the development of physical methods has been largely due to those with a background in physics and not chemistry. This has resulted in the emphasis being placed on the instrument and the study of molecular structure which has been very worth while and necessary. What is needed now is the application of these techniques to the study of analytical problems. Most of these are of a chemical nature, so that a background in chemistry and particularly physical chemistry is useful, even essential, in order to understand and interpret results properly. This, of course, must be combined with adequate training in physics, so that the limitations as well as the strength of any particular physical instrument for a particular analysis can be fully assessed.

We have never been accused of not supporting modern analytical instrumentation and we hope we never shall be, but some serious thought should be given to the basis of this growing feeling that, for example, to do gas analysis you must have a mass spectrometer, or to determine a metal a recording emission spectrometer is necessary. Developments in all fields extend rather than eliminate and yield more information faster on certain problems, but like all methods they have their limitations and sometimes they are serious ones.

Two industries where expensive analytical instrumentation has paid off are petroleum and metals. Here the product analyzed is reasonably constant, and interferences can be compensated for or standard samples can be prepared which closely parallel the composition of the unknown. In these industries continuous monitoring of the processes is also important; speed is essential, and, if possible, continuous recorded analysis is the preferred and ideal to which analytical research is directed. Under these circumstances chemical wet methods seem slow and cumbersome. The work which goes into the preparation of standards for such instrumental methods is considerable and is not justified for running a few samples. The basic methods used for standardization are, however, mostly chemical procedures and they provide a check by an independent method, which is the only way to get data on the validity of any method.

Analytical procedures, whether chemical or physical, may fail because of interferences or lack of sufficient sensitivity. Where this occurs in a physical method, a chemical separation of the interference is usually undertaken or the element is concentrated to the point where it can be detected. At this point the advantages of the physical approach are lost, because where concentration of the impurity is involved a chemical procedure may be more sensitive and easier to carry out. Present physical procedures largely deal with molecular properties peculiar to a particular substance and if the sample is diluted with unknown materials, false results can be obtained. A chemical separation and reaction with a color-forming substance can give an enormous leverage in detection and estimation, particularly if teamed with a spectrophotometric measurement of that color.

In our enthusiasm for modernity, let us not forget that the chemical procedures have a very important role to play in their own right, and when they are combined with the physical approach the sensitivity, specificity, and speed are greater than when each method is left to its own peculiar limitations.

Reaction of Carbon Disulfide with Primary and Secondary Aliphatic Amines as an Analytical Tool

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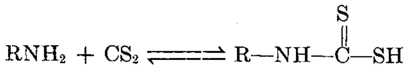
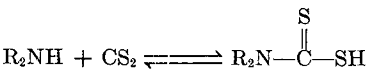
The reaction of primary and secondary amines with carbon disulfide to form dithiocarbamic acids is utilized as the basis of several analytical methods. In these methods the dithiocarbamic acids are titrated with standard aqueous sodium hydroxide using either phenolphthalein or thymolphthalein indicator. Methods are presented for the analysis of mixtures of primary, secondary, and tertiary aliphatic amines, and ammonia. For the analysis of these mixtures the reaction of primary amines with 2-ethylhexaldehyde to form the corresponding imine also has been utilized. Secondary amines do not undergo this reaction and are determined by conversion to the corresponding dithiocarbamic acids. The total primary and secondary amine content is determined by utilization of the carbon disulfide reaction. Tertiary amines and ammonia do not interfere in this procedure. By combining these methods with a tertiary amine and total amine determination a complete resolution of amine mixtures can be effected. Methods are also presented for the analysis of inorganic base-amine mixtures and acid-amine mixtures. These methods are of particular interest because a single sample is used. A method for the analysis of carboxylic acid–anhydride mixtures is described, which also uses a single sample. The anhydride in the sample is made to react with a measured excess of morpholine and gives one mole each of amide and acid. The acid formed plus any free acid present in the sample is titrated with standard sodium hydroxide to the thymolphthalein end point. The excess morpholine and the amide are neutral in the titration medium used. Excess morpholine is determined by conversion to the dithiocarbamic acid by reaction with carbon disulfide and titration with sodium hydroxide. From these two determinations the free acid and anhydride content of the sample are calculated. Potentiometric titration curves for the determination of several amines, inorganic base-amine mixtures, acid-amine mixtures, and carboxylic acid–anhydride mixtures are presented. Data obtained using indicators are presented for each method described. In general the accuracies obtained by the methods are within the order of ±0.1%. Interferences and compounds that fail to react are discussed.

IN THE methods presented here the reaction of primary and secondary amines with carbon disulfide to form dithiocarbamic acids has been utilized (*4*).



$$RNH_2 + CS_2 \rightleftharpoons R\text{—}NH\text{—}\overset{\overset{\displaystyle S}{\|}}{C}\text{—}SH \qquad (1)$$

and



$$R_2NH + CS_2 \rightleftharpoons R_2N\text{—}\overset{\overset{\displaystyle S}{\|}}{C}\text{—}SH \qquad (2)$$

Colorimetric methods of analysis for the determination of copper (*15*) and secondary amines (*10, 13*) have been based upon the ability of these acids to form highly colored copper chelates. Nebbia and Guerrieri (*10*) recently reported a titrimetric method for secondary amines which is based upon the formation of a nickel-dithiocarbamate complex. In these laboratories it was found that, under certain conditions, these dithiocarbamic acids can be titrated directly and quantitatively with standard sodium hydroxide. This principle has been utilized for the analysis of mixtures of aliphatic amines, inorganic base-amine, acid-amine, or carboxylic acid–anhydride.

DETERMINATION OF PRIMARY AND SECONDARY ALIPHATIC AMINES AND ANALYSIS OF AMINE-ACID AND AMINE-STRONG BASE MIXTURES

In general, the determination of primary and secondary amines in the presence of tertiary amines and ammonia has heretofore been accomplished by use of specific methods for the latter compounds. Acetylation procedures for tertiary amines (*8, 11, 14*) and the sodium cobaltinitrile method for ammonia (*9*) have been used. The primary and secondary amine content of the samples has been determined by difference. Recently McIntire, Clements, and Sproull (*7*) reported a colorimetric method, based upon the reaction of 1-fluoro-2,4-dinitrobenzene, for these amines. This section describes a specific method for the direct determination of the total primary and secondary amine content in the presence of tertiary amines. In this method an excess of carbon disulfide is reacted with the primary or secondary amine in an essentially nonaqueous medium such as isopropyl alcohol or a pyridine–isopropyl alcohol mixture. The dithiocarbamic acid formed in the reaction is then titrated with standard sodium hydroxide using phenolphthalein indicator. Potentiometric curves for the titration of the reaction products of carbon disulfide and three amines are shown in Figure 1. The method is unique in that primary and secondary amines are converted to acids and titrated with a base. Because of this fact, these amines can be determined in the presence of strong inorganic bases, ammonia, tertiary amines, and most acids.

APPARATUS AND REAGENTS

All potentiometric titrations were performed using a Leeds & Northrup line-operated pH meter equipped with glass and calomel electrodes.

Carbon disulfide, reagent grade.

Isopropyl alcohol, commercial grade, Carbide and Carbon Chemicals Co.

Pyridine, redistilled. This material should contain less than 0.0005 meq. of primary and secondary amines per gram. The amines are determined by the procedure described below.

Sodium hydroxide, 0.5*N*.

Hydrochloric acid, 0.5*N*.

Phenolphthalein, 1.0% pyridine solution.

Thymolphthalein, 1.0% pyridine solution.

PROCEDURE

To each of two 250-ml. glass-stoppered Erlenmeyer flasks, add the solvent specified in Table I. Reserve one of the flasks as a blank. Into the other flask introduce an amount of sample that contains not more than 15 meq. of primary or secondary amine. The sample aliquot should contain not more than 15 meq. of alkali or acid, and the total tertiary amine and ammonia content should not exceed 30 meq. If the sample contains alkali, it

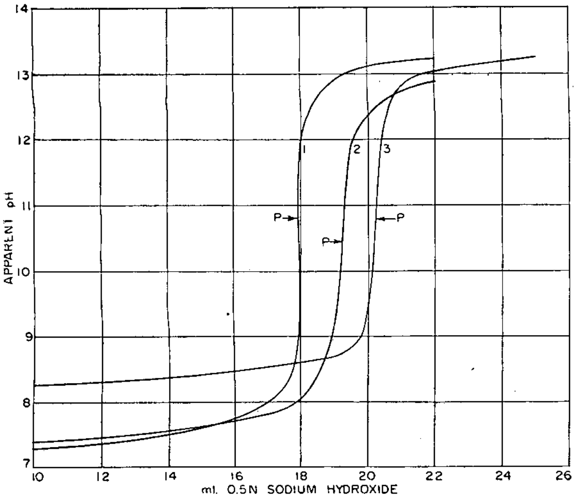


Figure 1. Potentiometric titration curves of dithiocarbamic acids from reactions of carbon disulfide with primary and secondary amines

1. 0.6771 gram of isopropanolamine, solvent C
2. 0.2868 gram of ethylenediamine, solvent A
3. 1.3085 grams of dibutylamine, solvent B

P. Phenolphthalein end point

should be neutralized with standard 0.5*N* hydrochloric acid using thymolphthalein indicator. For samples that contain acids, neutralize with standard 0.5*N* sodium hydroxide. For samples void of alkalies or acids, use phenolphthalein indicator. If more than 2.0 meq. of ammonia is present in the sample aliquot, cool the contents of the flasks to approximately −10° C. Carbon dioxide from a dry ice–organic solvent bath interferes in the subsequent titration; therefore, a brine bath is most convenient for this purpose.

By means of a pipet add 5 ml. of carbon disulfide to each flask and swirl to effect solution. Titrate the contents of each flask with 0.5*N* sodium hydroxide. For sample aliquots that contain more than 2.0 meq. of ammonia conduct this titration below 0° C., by placing the flask in a 1000-ml. beaker containing a mixture of crushed ice and methanol. Stir the contents of the flask by means of a magnetic stirrer to prevent a local excess of sodium hydroxide from accumulating in the titration medium. The end point selected should be the first definite pink color for phenolphthalein, or blue or blue-green color for thymolphthalein. The color should be stable at least 1 minute.

For samples that contain alkalies, the amount of hydrochloric acid consumed in the first titration is a measure of the alkali. For samples that contain acids the amount of sodium hydroxide necessary to neutralize the sample is a measure of the acid present. The amount of standard sodium hydroxide necessary to neutralize the sample after the addition of carbon disulfide is a direct measure of the primary and secondary amine content of the sample.

DISCUSSION

In the presence of a large excess of carbon disulfide the reaction of primary and secondary amines to give dithiocarbamic acids is approximately 90 to 95% complete. In general, secondary amines are more reactive than primary amines. To adapt this reaction to a quantitative method of analysis, it is forced to completion by means of the sodium hydroxide titrant. The potentiometric titration curves shown in Figure 1 were obtained by permitting the system to reach equilibrium before the addition of each increment of titrant. Usually equilibrium is established rapidly, except in the vicinity of the equivalence point.

An attempt was made to determine the dithiocarbamic acids by the addition of a measured excess of sodium hydroxide, and subsequent determination of the excess by titration with standard hydrochloric acid. Results obtained in this manner were erroneous due to the incompatibility of sodium hydroxide and carbon disulfide. In isopropyl alcohol medium and in the presence of excess sodium hydroxide, carbon disulfide reacts with the solvent to form a xanthate. Xanthate formation is even more pronounced when methyl or ethyl alcohol is substituted for isopropyl alcohol. Because of the tendency of isopropyl alcohol to react with carbon disulfide and sodium hydroxide, the end points obtained in this method are stable for only 4 or 5 minutes.

The reaction of carbon disulfide and primary and secondary amines cannot be forced to completion by heating because of the instability of the dithiocarbamic acids at elevated temperatures. In the case of alkyl amines, substituted ureas are formed by the evolution of hydrogen sulfide (*4*). Diamines, such as ethylenediamine, also liberate hydrogen sulfide but give polyalkylurea derivatives (*1*). Pyridine present in the reaction medium acts as a proton acceptor and tends to force the reaction of carbon disulfide with primary and secondary amines to completion.

Listed in Table I are several primary and secondary amines that have been determined successfully by this method. In each case the purity obtained by the carbon disulfide method is compared to the purity obtained by another acid-base titration method. Also listed are the solvent mixtures recommended for the determination of these amines. Solvent *A*, which contains 50 ml. of pyridine, 25 ml. of water, and 50 ml. of isopropyl alcohol, is used for the determination of amines that gave dithiocarbamic acids insoluble in isopropyl alcohol. Solvent *B*, isopropyl alcohol, is used for reactive amines that give soluble reaction products. Solvent *C*, which contains 75 ml. of isopropyl alcohol and 25 ml. of pyridine, is recommended for unreactive amines that give soluble reaction products. Of the primary and secondary amines investigated, only the aromatic amines and aliphatic amines that are highly branched in the 2 position, such as tertiary butylamine and diisopropylamine, do not react quantitatively under the conditions of the method.

Although the reaction of primary and secondary amines with carbon disulfide is specific, there is a slight tendency for ammonia to react with the reagent. The extent of the reaction is shown in Table II. In this table data for the determination of butylamine in the presence of ammonia are given. It can be seen that at temperatures below 0° C. the interference is negligible. At room temperature, however, the reaction is appreciable. In

Table I. Analysis of Primary and Secondary Amines by Reaction with Carbon Disulfide

Compound	Solvent Composition	Average Purity, Wt. %[a] Carbon disulfide method	Other[b]
2-Aminoethylethanolamine	A	100.0 ± 0.0 (2)	100.0 ± 0.1 (2)[c]
N-Aminoethylmorpholine	A	98.6 ± 0.1 (4)	98.8 ± 0.1 (2)[c]
Butylamine	B	97.9 (1)	97.5 ± 0.1 (2)
Butylamine, secondary	C	96.4 ± 0.1 (2)	96.4 (1)
Dibutylamine	B	100.1 ± 0.0 (2)	99.7 ± 0.0 (2)
Diethanolamine	C	99.8 ± 0.1 (2)	99.8 (1)
Diethylamine	B	97.4 ± 0.1 (3)	97.4 (1)
Diethylenetriamine	A	92.0 ± 0.0 (2)[d]	98.1 (1)[c]
Di(2-ethylhexyl)amine	C	100.0 ± 0.1 (2)	99.5 ± 0.1 (2)
Dihexylamine	B	93.4 (1)	93.4 ± 0.1 (2)
Dimethylamine, aqueous	B	39.6 ± 0.0 (2)	39.6 (1)
2,6-Dimethylpiperazine	A	98.9 ± 0.1 (2)	99.5 (1)[c]
Ethanolamine	C	99.3 ± 0.2 (3)	99.2 ± 0.2 (2)
Ethylamine, aqueous	B	70.2[e]	70.2 ± 0.1 (2)
Ethylenediamine	A	99.3 ± 0.2 (2)	99.2 (1)
2-Ethylhexylamine	C	99.0 (1)	98.7 (1)
Hexylamine	B	98.9 (1)	99.1 ± 0.0 (2)
Isobutylamine	B	99.3 ± 0.1 (2)	98.8 (1)
Isopropanolamine	C	99.2 ± 0.1 (2)	99.2 ± 0.0 (2)
Isopropylamine	C	99.1 ± 0.2 (2)	99.2 (1)
Methylamine, aqueous	B	44.9 ± 0.1 (2)	45.0 (1)
Morpholine	A	99.3 ± 0.1 (4)	99.4 (1)
Propylenediamine	A	99.0 ± 0.0 (2)	98.9 (1)

[a] Figures in parentheses represent number of determinations.

[b] By titration in water with standard 0.5*N* hydrochloric acid using bromocresol green–methyl red mixed indicator, unless otherwise specified.

[c] By titration in glacial acetic acid with standard 0.1*N* perchloric acid using crystal violet indicator.

[d] Sample contains 0.34 meq. of tertiary amine per gram.

[e] Standard deviation for 8 degrees of freedom is 0.11.

A = 50 ml. of pyridine, 25 ml. of water, and 50 ml. of isopropyl alcohol.
B = 75 ml. of isopropyl alcohol. If more than 2.0 meq. of ammonia is present, add 25 ml. of pyridine.
C = 25 ml. of pyridine and 75 ml. of isopropyl alcohol.

Table II. Determination of Butylamine in Aqueous Ammonia Solutions

(Extent of ammonia interference)

Sample[a]	Added: Ammonia	Added: Butylamine	Butylamine Found	Deviation
Ammonia-butylamine	27.00	6.73	6.79[b]	+0.06
	23.50	18.85	18.95[b]	+0.10
	19.40	32.60	33.10[b]	+0.50
Ammonia	29.00	0.00	0.00[b]	0.00
	2.90	0.00	0.00[c]	0.00
	5.80	0.00	0.28[c]	+0.28
	29.00	0.00	0.73[c]	+0.73

[a] All values are per cent by weight.
[b] Reaction with carbon disulfide and subsequent titration at <0° C.
[c] Reaction with carbon disulfide and subsequent titration at 25° C.

general, the reaction is more pronounced in pyridine medium than in isopropyl alcohol. For this reason pyridine alone is not used as a solvent for the reaction. Even in the absence of ammonia it is necessary to use either isopropyl alcohol or water as a cosolvent to solubilize the sodium hydroxide in the titrant. As stated previously, methanol cannot be used because of xanthate formation. The maximum amount of ammonia that can be tolerated is given under Procedure.

Tertiary amines do not react with carbon disulfide. In the method described here large quantities of pyridine (a tertiary amine) have been used as a cosolvent. The extent of interference from tertiary amines is determined by the basicity of the amine in the titration medium. Under the conditions of the method as much as 30 meq. of most tertiary amines will not interfere. Tertiary amines and ammonia are more basic in isopropyl alcohol than in pyridine–isopropyl alcohol mixtures. When large quantities of tertiary amines or ammonia are present it is sometimes necessary to add sufficient pyridine to suppress the basicity of these compounds.

Because the method is based upon an alkalimetric titration, compounds that are acidic or basic under the conditions of the titration will interfere. Acids with ionization constants greater than approximately 1×10^{-7} in water and bases with ionization constants greater than about 1×10^{-2} interfere quantitatively. Figure 2 shows a potentiometric titration of an aqueous mixture of sodium hydroxide and morpholine. The curve was obtained by first titrating the sodium hydroxide with standard hydrochloric acid. After this equivalence point was obtained carbon disulfide was added and the dithiocarbamic acid formed by reaction with morpholine was titrated with standard sodium hydroxide. The amount of morpholine present in the sample was calculated from the total amount of sodium hydroxide titrant added minus the hydrochloric acid titrant added in excess of the first equivalence point. Table III shows data for the analysis of known mixtures of morpholine and sodium hydroxide. These data were obtained in the same manner as described above, except that thymolphthalein indicator was used and the titration of sodium hydroxide with hydrochloric acid was not extended beyond the end point. Although other methods are available for the analysis of mixtures of this type, the method described is of merit because a single sample is sufficient for both determinations.

Mixtures of acids and primary and/or secondary amines can be analyzed by a procedure similar to that described above. Figure 3 shows a potentiometric titration of a mixture of morpholine and hydrochloric acid. The hydrochloric acid present in the sample was titrated with standard sodium hydroxide. After the end point was obtained, carbon disulfide was added and the titration of the dithiocarbamic acid was performed. The data in Table IV, for the analysis of morpholine–hydrochloric acid mixtures and dibutylamine–acetic acid mixtures, were obtained in this manner, except that thymolphthalein indicator was used. This method should be of considerable advantage for the analysis of amine-acid mixtures because the use of a single sample makes the method rapid and convenient.

By combining the method presented here with a total base and a tertiary amine determination it is possible to determine indirectly the amount of ammonia present in a particular sample. A further discussion of this is given below.

ANALYSIS OF MIXTURES OF AMMONIA, PRIMARY, SECONDARY, AND TERTIARY ALIPHATIC AMINES

The analysis of mixtures of amines and ammonia is a difficult analytical problem that confronts any manufacturer or consumer of amines. The determination of tertiary amines by acetylation (*8, 11, 14*) and of ammonia by the sodium cobaltinitrite method (*9*) is satisfactory for these classes of compounds. However, for the complete resolution of amine mixtures a method of analysis must be available for the specific determination of either primary or secondary amine. The salicylaldehyde method of Siggia, Hanna, and Kervenski (*12*) and the benzaldehyde

Table III. Analysis of Aqueous Morpholine–Sodium Hydroxide Mixtures of Known Composition by Carbon Disulfide Reaction

Added[a]: Morpholine	Added[a]: NaOH	Found[a]: Morpholine	Found[a]: NaOH	Deviation[a]: Morpholine	Deviation[a]: NaOH
34.2	2.79	34.0	2.80	+0.20	+0.01
16.0	3.56	16.0	3.57	0.00	+0.01
31.9	2.89	32.0	2.94	+0.10	+0.05
15.9	3.57	16.0	3.55	+0.10	−0.02
3.60	4.04	4.67	4.02	+0.07	−0.02
0.48	4.22	0.50	4.19	+0.02	−0.03

[a] All values are per cent by weight.

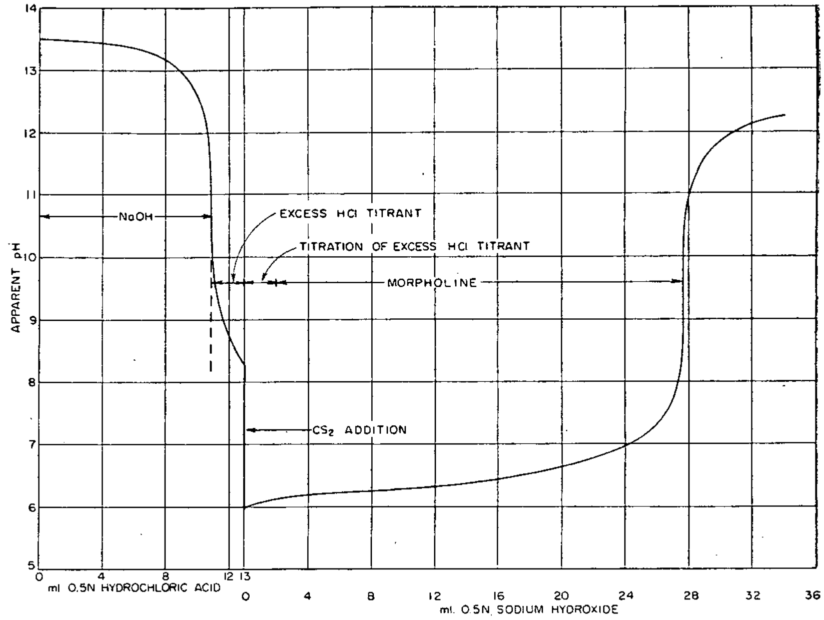


Figure 2. Potentiometric titration curve for determination of a mixture containing 3.46% sodium hydroxide and 18.5% morpholine

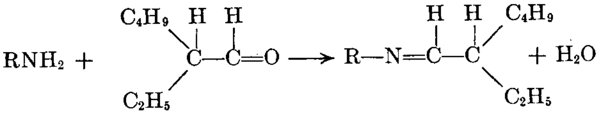
procedure of Hawkins, Smith, and Mitchell (*3*) have been used for the determination of secondary and tertiary amines in the presence of primary amines. Ammonia interferes in these methods and must be removed. A modification of the salicylaldehyde method used in these laboratories is specific for certain primary amines, although ammonia interferes (*5*). Recently Nebbia and Guerrieri (*10*) reported a titrimetric method specific for secondary amines which is based upon the formation of a nickel dithiocarbamate by reaction of secondary amines with carbon disulfide in the presence of ammonia and nickel ion. These authors also reported a colorimetric method for secondary amines based upon the highly colored copper dithiocarbamates. A colorimetric method for primary amines based upon the copper chelates of salicylaldehyde imines was recently developed by the authors of this paper (*2*). Ammonia also interferes in this method.

Table IV. Analysis of Aqueous Amine-Acid Mixtures of Known Composition by Carbon Disulfide Reaction

Sample	Added[a]		Found[a]		Deviation[a]	
	Acid	Amine	Acid	Amine	Acid	Amine
Morpholine–hydrochloric acid	2.32	34.50	2.39	33.90	+0.07	−0.60
	2.92	17.60	2.97	16.40	+0.05	−0.20
	3.37	4.81	3.40	4.78	+0.03	−0.03
	3.51	0.84	3.52	0.85	+0.01	+0.01
Dibutylamine–acetic acid	38.80	61.20	38.50	61.80	−0.30	+0.60
	10.20	89.80	10.20	89.80	0.00	0.00
	87.60	12.40	87.70	12.60	+0.10	+0.20

[a] All values are per cent by weight.

A specific method for primary and secondary amines has just been described in which the amines are reacted with carbon disulfide and the dithiocarbamic acids formed are titrated with standard sodium hydroxide. Ammonia and tertiary amines do not interfere in this method. In the method presented here primary amines are reacted with 2-ethylhexaldehyde to form the corresponding imine.



$$RNH_2 + \underset{C_2H_5}{\overset{C_4H_9\ \ H\ \ H}{>C{-}C{=}O}} \longrightarrow R{-}N{=}\overset{H\ \ H\ \ C_4H_9}{C{-}C}\underset{C_2H_5}{<} + H_2O \quad (3)$$

Secondary amines do not react with the aldehyde and are converted to the corresponding dithiocarbamic acids as shown in Equation 2. The imines from the primary amines do not react with carbon disulfide to form acids and therefore do not interfere. The dithiocarbamic acids are titrated, at reduced temperature and in organic medium, with standard sodium hydroxide using phenolphthalein indicator. The imines formed according to Equation 3, ammonia, and tertiary amines are not basic under these conditions and do not interfere.

REAGENTS

Carbon disulfide, reagent grade.

Isopropyl alcohol, Carbide and Carbon Chemicals Co., commercial grade.

Pyridine, redistilled. This material should not contain more than 0.0002 meq. of primary and secondary amines per gram.

2-Ethylhexaldehyde, 50% by volume pyridine solution containing approximately 0.5% phenyl-1-naphthylamine inhibitor. The 2-ethylhexaldehyde used was Carbide and Carbon Chemicals Co. commercial grade. The reagent was stored in brown bottles and discarded when the blank described below exceeded 0.5 ml.

Phenolphthalein, 1.0% pyridine solution.

Sodium hydroxide, 0.5*N*.

PROCEDURE

Pipet 10.0 ml. of the 2-ethylhexaldehyde solution into each of two 250-ml. glass-stoppered Erlenmeyer flasks. If specified in Table V add 50 ml. of isopropyl alcohol to each flask. Reserve one of the flasks as a blank. Into the other flask introduce an amount of sample that contains not more than 13 meq. of secondary amine. The combined ammonia and tertiary amine content of the sample aliquot should not exceed 30 meq. The primary amine content of the sample aliquot should be less than 16 meq. Allow the sample and blank to stand at room temperature for 5 minutes and add the solvent mixture specified in Table V. Cool the contents of the flask to −10° ± 2° C. using a suitable bath. Do not use dry ice–organic solvent baths because carbon dioxide interferes in the titration. Remove the flasks from the bath and add 5 ml. of carbon disulfide by means of a pipet. Add 5 or 6 drops of phenolphthalein indicator to each flask and immediately titrate with standard 0.5*N* sodium hydroxide at below 0° C. This is best done by placing the flask in a 1000-ml. beaker containing a slurry of crushed ice and methanol. Stir the contents of the flask by means of a magnetic stirrer. The end point selected should be the first definite pink color stable for at least 1 minute.

DISCUSSION

It has already been mentioned that most aliphatic secondary amines react with carbon disulfide; under the conditions of the first method described, they can be quantitatively titrated as the dithiocarbamic acids. Aromatic amines and highly branched amines such as diisopropylamine cannot be titrated in this manner. The limiting factor in this method for the determination of secondary amines in the presence of primary amines is the ability to destroy the primary amine quantitatively. Several aldehydes were investigated for this purpose. Any aldehyde suitable for use in this method must have the following characteristics: (*a*) be resistant to sodium hydroxide; (*b*) be resistant to oxidation; (*c*) react with primary amines to form imines; and (*d*) not react with secondary amines. Salicylaldehyde meets the above requirements satisfactorily, but can not be used in the method because it is acid to phenolphthalein. Benzaldehyde satisfies conditions *a*, *b*, and *d*, but is easily oxidized to benzoic acid, thus causing unstable blanks. Lower aliphatic aldehydes, such as formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde, tend to react with secondary amines. Of all the aldehydes investigated, 2-ethylhexaldehyde was found best suited for use in this method (isobutyraldehyde is less effective than ethylhexaldehyde for reaction of primary amine). The aldehyde is sufficiently resistant to sodium hydroxide titrant. Although autoxidation occurs to a slight extent, it can be inhibited by the addition of phenyl-1-naphthylamine to the aldehyde reagent. In one instance an apparent reaction of 2-ethylhexaldehyde with a secondary amine was noted, giving low results. For the determination of dimethylamine in the

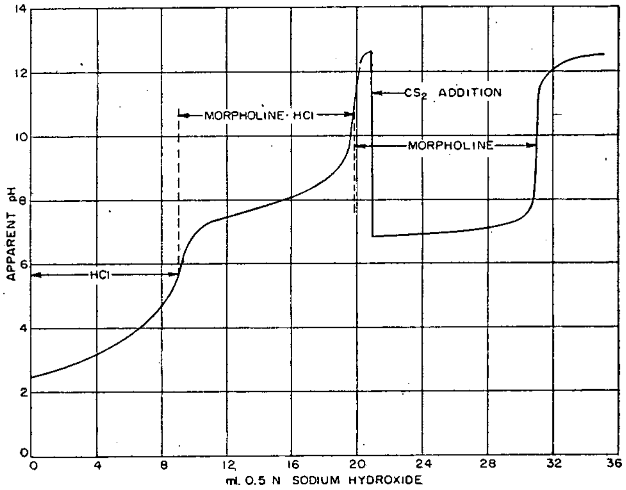


Figure 3. Potentiometric titration curve for determination of a mixture containing 3.39% hydrochloric acid and 4.56% morpholine

Table V. Determination of Secondary Amines by Use of 2-Ethylhexaldehyde–Carbon Disulfide Method

Compound	Solvent Mixture	Purity, Wt. %[a] Carbon disulfide method	Purity, Wt. %[a] Other[b]
Dibutylamine	B	99.6 ± 0.0 (2)	99.7 ± 0.0 (2)
Diethylamine	B	96.8[c]	97.4 (1)
Di(2-ethylhexyl)amine	C	98.6 ± 0.1 (2)	99.5 ± 0.1 (2)
Dihexylamine	B	92.7 (1)	93.4 ± 0.0 (2)
Dimethylamine, aqueous	D	38.6 ± 0.05 (3)	39.6 (1)
2,6-Dimethylpiperazine	A	98.8 ± 0.05 (2)	99.5 (1)[d]
Morpholine	A	99.3 ± 0.1 (2)	99.4 (1)

[a] Figures in parentheses represent number of determinations.

[b] By titration with standard hydrochloric acid to bromocresol green–methyl red mixed indicator unless otherwise indicated.

[c] Standard deviation for 6 degrees of freedom is 0.16.

[d] By titration with standard perchloric acid in acetic acid using crystal violet indicator.

A = 50 ml. of pyridine, 25 ml. of water, and 50 ml. of isopropyl alcohol.
B = 75 ml. of isopropyl alcohol. If more than 2 meq. of ammonia is present, add sufficient pyridine to suppress its basicity.
C = 75 ml. of isopropyl alcohol and 25 ml. of pyridine.
D = Reaction with 2-ethylhexaldehyde in presence of 50 ml. of isopropyl alcohol. Add 25 ml. isopropyl alcohol before reaction with carbon disulfide.

presence of methylamine, it was necessary to carry out the reaction with 2-ethylhexaldehyde in 50 ml. of isopropyl alcohol to inhibit the reaction with dimethylamine. In all other cases the reaction takes place with 10 ml. of 50% by volume of the aldehyde in pyridine.

2-Ethylhexaldehyde reacts quantitatively with most simple aliphatic primary amines to form the corresponding imine. A 100% excess of the aldehyde is sufficient to force this reaction to completion. Using a 50% excess of the aldehyde high results were obtained for the determination of dimethylamine in the presence of methylamine, thus indicating an insufficient excess of aldehyde reagent. When a 10-ml. aliquot of the reagent is used, which contains approximately 32 meq. of aldehyde, no more than 16 meq. of primary amine should be present in the sample aliquot used for analysis. This aldehyde does not react quantitatively with primary alcohol amines, aromatic amines, primary amines highly branched in the 2 position such as *tert*-butylamine and isopropylamine, and polyamines such as ethylenediamine. When the above amines are present they interfere in the determination of secondary amines by this method.

Table V lists several secondary amines that have been successfully determined by this method. Only the secondary amines, whose corresponding primary amines react quantitatively with 2-ethylhexaldehyde, are listed in this table. Secondary amines for which no corresponding primary amine exists are also included. The purity obtained by the 2-ethylhexaldehyde–carbon disulfide method is compared with that obtained by a total base titration. The data obtained by the former method are usually slightly lower than those obtained by the latter; this is no doubt caused by the presence of small amounts of primary and tertiary amines in these samples. Also included in this table is the solvent mixture recommended for each amine. The effect of the solvent upon the reaction of these amines with carbon disulfide has already been discussed.

Data are listed in Table VI for the analysis of several mixtures of primary and secondary amines of known composition. In a few cases both the primary and the secondary amine content of the samples was obtained. For these analyses the total primary and secondary amine content was determined by titration of the corresponding dithiocarbamic acids of these amines. Secondary amines were determined in these samples by the procedure described in this section, and the primary amine content was obtained by difference.

By using this method for the specific determination of secondary amines and the carbon disulfide method for primary and secondary amines, combined with a total base and a tertiary amine determination, it is possible to resolve certain amine mixtures completely. Listed in Table VII are data on the analysis of known mixtures of ammonia and primary, secondary, and tertiary amines. The data were obtained as described above. For the determination of the tertiary amine content the samples were reacted with acetic anhydride in methanol; the tertiary amine was then titrated with alcoholic hydrochloric acid using methyl yellow–methylene blue mixed indicator. The total amine content was determined by titration in water with standard hydrochloric acid and bromocresol green–methyl red mixed indicator.

Because this method is based upon an alkalimetric titration of a dithiocarbamic acid, materials that are acidic or basic under the condition of the titration interfere. The maximum amounts of ammonia and tertiary amines that can be tolerated are given under Procedure in the section on the 2-ethylhexaldehyde–carbon disulfide method. A more detailed discussion of interferences is in the section on the determination of primary and secondary amines.

This method should be of considerable use for the analysis of mixtures of simple aliphatic amines and for the determination of purities of secondary amines. Mixtures of amines and ammonia can be analyzed without resorting to a specific removal or determination of ammonia. Even in the presence of large concentrations of ammonia, certain amine mixtures can be completely resolved. The lower limit of determination of ammonia, using the two carbon disulfide methods with a tertiary amine and a total base determination, is limited because ammonia is obtained by difference.

DETERMINATION OF CARBOXYLIC ACIDS AND ANHYDRIDES BY MORPHOLINE–CARBON DISULFIDE METHOD

A satisfactory review of methods available for the analysis of carboxylic acid–anhydride mixtures was recently given in a paper by Johnson and Funk (*6*). The method presented by these authors is based upon the reaction of morpholine with an anhydride to give one mole each of acid and amide. In this method a known excess of morpholine is added and the excess is determined by a nonaqueous titration. For the determination of free acid, a total acid value must be obtained by an independent procedure.

The method presented here was made possible by the discovery that primary and secondary amines can be titrated as the corresponding dithiocarbamic acids. In the method finally adopted, the sample containing anhydride is added to a measured excess of morpholine solution. The free acid present in the sample and the acid formed by reaction of anhydride are titrated with standard sodium hydroxide. Under the conditions of the titration

Table VI. Analysis of Known Mixtures of Primary and Secondary Amines by Use of 2-Ethylhexaldehyde–Carbon Disulfide Method

Mixture	Added, % by Wt. Primary	Added, % by Wt. Secondary	Found, % by Wt.[a] Primary	Found, % by Wt.[a] Secondary	Deviation Primary	Deviation Secondary
Methylamine	19.4	22.3	19.4 ± 0.03 (2)	22.3 ± 0.06 (5)	0.0	0.0
Hexylamines	25.6	65.6	26.0	64.8	+0.4	−0.8
2-Ethylhexylamines	79.8	19.4	....	19.1	...	−0.3
	39.0	60.0	....	60.4	...	+0.4
	49.6	49.9	....	50.2	...	+0.3
Butylamines	47.4	51.8	47.1 ± 0.05 (3)	51.8 ± 0.05 (3)	−0.3	0.0
	61.2	38.5	....	61.0	...	−0.2
	28.7	69.5	....	28.9	...	+0.2
	69.6	30.2	....	69.4	...	−0.2

[a] Single determinations unless otherwise indicated by figures in parentheses.

the excess morpholine does not interfere. The addition of carbon disulfide at the equivalence point of this titration converts the excess morpholine to the corresponding dithiocarbamic acid. This acid is then titrated with standard sodium hydroxide. The difference between a blank and a sample for the second titration is a measure of the anhydride originally present. The difference between the two determinations, in milliequivalents per gram, is a measure of the free acid present in the sample. A potentiometric titration curve for the determination of acetic anhydride by this method is given in Figure 4.

Table VII. Analysis of Known Amine Mixtures by Use of 2-Ethylhexaldehyde–Carbon Disulfide Method

Mixture	Compound	% by Weight Added	% by Weight Found	Deviation
No. 1	Ammonia	5.5	5.2	−0.3
	Butylamine	19.8	19.9	+0.1
	Dibutylamine	20.9	21.1	+0.2
	Tributylamine	16.6	16.7	+0.1
No. 2	Ammonia	0.9	0.7	−0.2
	Butylamine	25.7	26.0	+0.3
	Dibutylamine	38.8	38.9	+0.1
	Tributylamine	8.1	8.3	+0.2
No. 3	Ammonia	1.8	1.0	−0.8
	Ethylamine	26.0	26.4	+0.4
	Diethylamine	43.8	43.2	−0.6
	Triethylamine	21.6	22.3	+0.7

REAGENTS

Carbon disulfide, reagent grade.

Isopropyl alcohol, commercial grade, Carbide and Carbon Chemicals Co.

Morpholine, 0.2N in acetonitrile. Transfer 17 ml. of morpholine to a 1000-ml. volumetric flask and dilute to volume with acetonitrile, Carbide and Carbon Chemicals Co. commercial grade.

Thymolphthalein indicator, 1.0% pyridine solution.

Sodium hydroxide, 0.1N.

PROCEDURE

Pipet 25 ml. of the 0.2N morpholine solution into each of two 250-ml. glass-stoppered Erlenmeyer flasks. Reserve one of the flasks as a blank. Into the other flask introduce an amount of sample that contains between 2.0 and 3.5 meq. of acid anhydride and acid. Allow both the sample and blank to stand at room temperature for 15 minutes. To each flask add 75 ml. of isopropyl alcohol, followed by 5 or 6 drops of thymolphthalein indicator. Titrate with 0.1N sodium hydroxide to the first blue color stable for at least 15 seconds. Record these titrations and zero the buret for both the sample and the blank. Do not overtitrate the end point. Add 20 ml. of water to the blank. Pipet 5 ml. of carbon disulfide into each flask and swirl to mix the contents thoroughly. Titrate the contents of each flask with 0.1N sodium hydroxide. Swirl the flasks during the titration to prevent a local excess of titrant in the titration medium. The end point selected should be the first blue or blue-green color stable for at least 1 minute. The difference between the blank and sample for the second titration is a direct measure of the anhydride originally present. The difference between the first determination, and the second, in milliequivalents per gram, is a measure of the free acid in the sample. The free acid can also be calculated from the difference between the total volumes of sodium hydroxide used for the sample and the blank.

DISCUSSION

The potentiometric titration curves shown in Figure 4 were obtained by a procedure similar to that just described. For the titration of both the sample and the blank, carbon disulfide was added after the titration curve for the first equivalence point was obtained. In the procedure described above thymolphthalein indicator is specified. In this case carbon disulfide is added at the equivalence point of the first titration. For the curves in Figure 4 the difference between the sample and the blank, for the first titration, is a measure of the free acetic acid and acetic anhydride present in the sample. For the sample titration curve, S, the difference between the first equivalence point and the second is a measure of excess morpholine. The difference between the amount of sodium hydroxide consumed in the titration of the second equivalence point of the blank, curve B, and the excess morpholine for the sample, is a measure of acetic anhydride. Using the indicator technique, the calculations are simplified by leveling the buret at zero after the titration to the first end point. By this procedure the first titration is obtained by subtracting the blank from the sample; the second titration calculation is the blank minus the sample. Point T in Figure 4 represents the thymolphthalein indicator end point. Although the indicator end point is slightly higher than the potentiometric end point the error introduced is nearly the same for both the sample and the blank. The use of phenolphthalein is not recommended because the difference in the potentiometric and indicator end points for the first titration is too large.

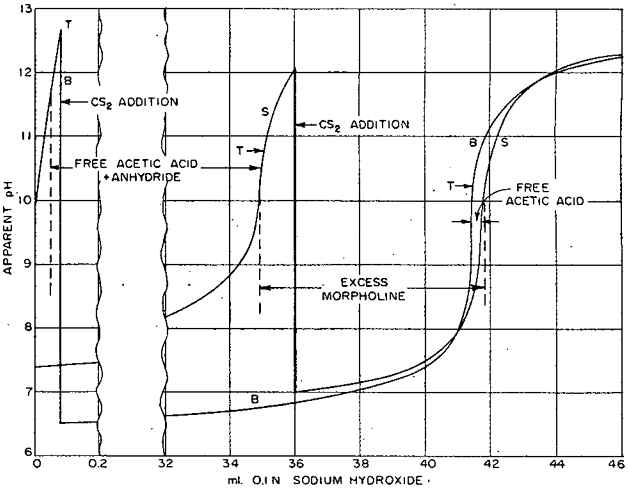


Figure 4. Potentiometric titration curves for determination of acetic anhydride by morpholine–carbon disulfide method

B = blank
S = sample
T = thymolphthalein end point

In the method presented here 0.2N morpholine in acetonitrile and 0.1N sodium hydroxide titrant are specified. The titration is carried out using 75 ml. of isopropyl alcohol as a cosolvent. To prevent precipitation of the dithiocarbamic acid on the addition of carbon disulfide, a small amount of water must be incorporated in the medium. Sufficient water is introduced by the titrant for the titration of the sample, but in the case of the blank, 20 ml. of water must be added before the addition of carbon disulfide. Stronger reagents such as 1N morpholine and 0.5N sodium hydroxide cannot be used if a procedure similar to the above is employed. On the addition of water in the titrant to a titration medium consisting of 25 ml. of 1N morpholine in acetonitrile and 75 ml. of pyridine, morpholine becomes basic to thymolphthalein indicator. Also the dithiocarbamic acid formed on the addition of carbon disulfide cannot be solubilized by water. Satisfactory results have been obtained with these stronger reagents when the procedure is modified by preparing the morpholine reagent using pyridine as the solvent and using a titration medium consisting of 50 ml. of pyridine, 25 ml. of water, and 50 ml. of isopropyl alcohol.

Listed in Table VIII are several anhydrides for which the purities were determined using the method just described. Data are also shown for the determination of the free acid present in each of these samples. In most cases the purities are compared

Table VIII. Purity of Anhydrides by Morpholine–Carbon Disulfide Method

	Purity, % by Wt.[a]			Total, % by Wt.	
Anhydride	Morpholine–carbon disulfide	Morpholine[a]	Acid Content, % by Wt.[a]	Morpholine–carbon disulfide	Morpholine[b]
Acetic	98.8 ± 0.1 (4)	98.9	0.4 ± 0.1 (4)	99.2	99.3
Butyric	97.3 ± 0.2 (2)	96.9	2.7 ± 0.1 (2)	100.0	99.6
Chrysanthemum	97.4 ± 0.2 (2)	97.8	1.6 ± 0.0 (2)	99.0	99.4
Endomethylenetetrahydrophthalic (Carbic)	96.0 ± 0.0 (2)	..	1.5 ± 0.0 (2)	97.5	...
2-Ethylhexanoic	90.9 ± 0.2 (2)	91.1	8.9 ± 0.0 (2)	99.8	100.0
Maleic	98.2 ± 0.1 (4)	..	0.9 ± 0.03 (4)	99.1	...
3-Methylglutaric	98.5 ± 0.05 (2)	99.5	1.5 ± 0.05 (2)	100.0	101.0
Phthalic	99.5 ± 0.2 (2)	99.4	0.6 ± 0.05 (2)	100.1	100.0
Propionic	96.5 ± 0.1 (2)	96.9	3.3 ± 0.0 (2)	99.8	100.2
Succinic	95.7 ± 0.1 (2)	95.2	4.4 ± 0.05 (2)	100.1	99.6

[a] Figures in parentheses indicate number of determinations.
[b] Procedure of Johnson and Funk (*6*).

Table IX. Analysis of Mixtures of Anhydrides and Their Corresponding Acids by Morpholine–Carbon Disulfide Method

	Added, % by Wt.			Found, % by Wt.		
Acid-Anhydride Mixture	Acid	Anhydride	Total	Acid	Anhydride	Total
Maleic	25.4	74.0	99.4	25.7	74.3	100.0
	53.6	46.0	99.6	53.7	46.0	99.7
Acetic	53.8	45.5	99.3	53.9	45.4	99.3
	21.7	77.6	99.3	21.9	77.7	99.6

with results obtained using the method of Johnson and Funk (*6*). The precision is in the order of ±0.1% for the determination of purity. In Table IX the analyses of several known mixtures of carboxylic acids and their corresponding anhydrides are listed. For these data the accuracy of the method is within ±0.2% for both the acid and the anhydride determinations.

In general, the method is subject to the same interferences as the method of Johnson and Funk (*6*), except that organic acids interfere quantitatively and tertiary amines are not basic to the indicator. The method is applicable to the determination of a wide variety of anhydrides. Maleic anhydride can be determined by the method if $0.2N$ morpholine solution is used. With $1.0N$ morpholine, addition across the double bond occurs. The method of Johnson and Funk (*6*) cannot be used for this anhydride because maleic acid is acidic to the indicator used in this method. Of the compounds investigated only acrylic and chloroacetic anhydride could not be determined by this method. Halogen-substituted anhydrides react with morpholine to liberate a halogen acid; acrylic compounds add morpholine across the unsaturation. The method also has merit in that both the acid and anhydride determinations are obtained using a single sample and titrant.

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Received for review October 21, 1955. Accepted January 10, 1956.

Colorimetric Determination of Primary Aliphatic Amines by Copper-Salicylaldehyde Method

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A new method for the determination of primary aliphatic amines in the presence of secondary and tertiary amines is described. The primary amine is made to react with an aqueous reagent containing cupric chloride, salicylaldehyde, and triethanolamine. The copper-salicylaldehydeimine formed in this reaction is extracted into 1-hexanol. The amount of copper in the hexanol layer, which is determined colorimetrically by reaction with bis(2-hydroxyethyl)dithiocarbamic acid, is a measure of primary amine in the sample. The method has been successfully applied to 15 amines. Data for the analysis of several amine mixtures are given. In most cases an accuracy within 5% can be expected. Interfering compounds and those which fail to react are discussed.

In the difficult analytical problem of separating primary and secondary amines, the method of Van Slyke (*7*) is possibly the most generally used for the determination of primary amino nitrogen. Because this procedure is very time-consuming and requires an experienced analyst to obtain precise data, the applicability of the method is limited. Hawkins, Smith, and Mitchell (*3*) describe a method for primary amines by reaction with benzaldehyde. By this procedure the water liberated in the reaction is measured by the Karl Fischer reagent in the presence of hydrogen cyanide. This toxic reagent often precludes the use of this procedure for routine control analyses. The reaction of salicylaldehyde with primary amines to form the imine was used by Wagner, Brown, and Peters (*8*) as the basis of a method for the analysis of amine mixtures. This procedure has been modified by Siggia, Hanna, and Kervenski (*6*) to include aromatic amines. In these procedures secondary and tertiary amines are titrated in nonaqueous media. Because of the poor end points obtained for these titrations it is necessary to determine the entire potentiometric titration curve for each sample. In an indicator modification of the above procedure, which has been devised and used successfully in these laboratories (*4*), a measured excess of salicylaldehyde is made to react with the amine in pyridine medium. The excess salicylaldehyde, which is determined by titrating with sodium methylate in pyridine using phenolphthalein indicator, is a measure of primary amine present in the sample. This method is applicable principally to simple aliphatic amines.

An investigation was undertaken, therefore, to develop a general method of analysis for primary amines in the presence of secondary and tertiary amines to replace the Van Slyke procedure, which is used as a control method in these laboratories. The qualitative test of Duke (*1*) was investigated as a possible quantitative procedure. The basis of this test is the reaction of a primary amine with 5-nitrosalicylaldehyde. The imine formed in this reaction reacts with nickel ion in the presence of triethanolamine to form an insoluble compound.

DEVELOPMENT OF REAGENT

In attempting to adapt the reagent specified by Duke to a quantitative basis, it was found that nickel-5-nitrosalicylaldehyde derivatives of the primary amines were insoluble in most solvents and could not be determined colorimetrically. By substituting copper for nickel and salicylaldehyde for the corresponding 5-nitro compound, derivatives soluble in certain organic solvents were obtained. A reagent containing salicylaldehyde, cupric acetate, and triethanolamine was prepared in methanol. With this reagent primary amines give a soluble, colored reaction product which has an absorption maximum at 445 mμ and follows Beer's law. Secondary amines interfere with this reagent because they also form a colored reaction product. Because of this interference an aqueous reagent was prepared, in which most of the reaction products of primary amines are insoluble. Reaction products of primary amines can be extracted into isopropyl ether or benzene; however, when this technique is used, the colored reaction products have no absorption maximum in the visible portion of the spectrum.

An attempt was made to utilize the procedure in spite of this limitation by measuring the absorbance at 430 mμ, which is on the shoulder of the curve. A calibration curve, which was linear except near the origin, was obtained by this procedure. The addition of 0.01% ethanolamine to the triethanolamine used in the reagent gave a calibration curve that followed Beer's law over all ranges of concentration. However, secondary and tertiary amines cause a shift in the absorption curve. Because of this effect it was necessary to develop a color which possesses an absorption maximum in the visible portion of the spectrum independent of secondary or tertiary amines.

An aliquot of the benzene layer was removed and the copper present made to react with diethyldithiocarbamic acid (*2*). The absorbance at 430 mμ is a direct measure of copper and, therefore, an indirect measure of primary amine. Fair results were obtained by this method for the determination of primary amines in the presence of secondary and tertiary amines. However, there were three serious disadvantages: (*a*) The amount of monoethanolamine present in the triethanolamine used in the reagent determined the slope of the calibration curve; (*b*) low results were obtained for the analysis of alcoholamines in the presence of the corresponding secondary and tertiary amines; and (*c*) the presence of monoethanolamine in the reagent caused the reagent blank to have a high absorbance.

Because of these disadvantages, several organic solvents were investigated for the extraction of the copper complexes. The use of 1-hexanol eliminated the necessity of adding monoethanolamine to the reagent. A superior color stability was also imparted by substituting bis(2-hydroxyethyl)dithiocarbamic acid for diethyldithiocarbamic acid as the reagent for copper (*9*).

Effect of Triethanolamine Concentration. Triethanolamine is used in the reagent to act as a proton acceptor to shift the equilibrium reaction between cupric chloride and the imine. A minimum of 15.0% by volume of the amine is necessary to give quantitative reaction. The minimum amount of triethanolamine is specified in the reagent to allow for the presence of secondary and tertiary amines in the sample.

Effect of Salicylaldehyde Concentration. The compatibility of cupric ion and salicylaldehyde is limited by the formation of an insoluble complex. Fortunately, in dilute solutions and in the presence of triethanolamine, this chelate formation is minimized. A maximum of 0.5% by volume of salicylaldehyde can be tolerated in the reagent without appreciable reaction with cupric ion. This concentration has been specified in the reagent.

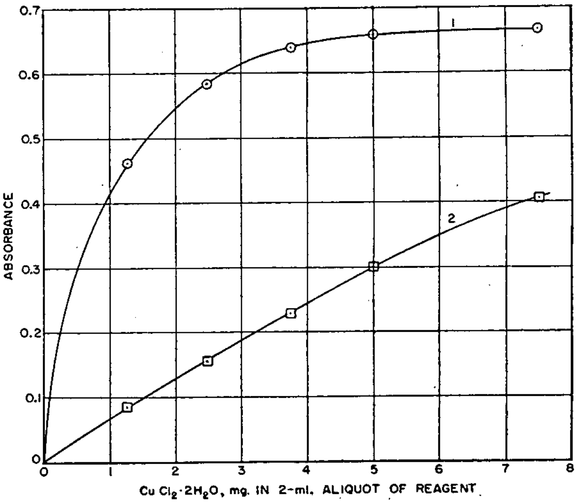

Figure 1. Effect of copper concentration on reaction of copper-salicylaldehyde reagent with ethanolamine

1. Absorbance for 0.372 mg. of ethanolamine
2. Absorbance of reagent blank *vs.* methanol

Effect of Copper Concentration. The concentration of cupric ion in the reagent is fairly critical, as shown in Figure 1. Curve 1 is a plot of the absorbance obtained for 0.372 mg. of ethanolamine as a function of the concentration of cupric chloride dihydrate in 2.0 ml. of reagent. The concentrations of triethanolamine and salicylaldehyde are fixed at 15.0 and 0.5% by volume, respectively. From this curve it can be seen that at least 5.0 mg. of cupric chloride dihydrate is necessary to obtain maximum reaction. Curve 2 shows the effect of cupric ion on the absorbance of the reagent blank compared to methanol. From this curve it is apparent that a minimum concentration of cupric ion is desired to obtain a minimum absorbance of the reagent blank. For this reason 5 mg. of cupric chloride dihydrate in a 2-ml. aliquot of the reagent is specified. This corresponds to a cupric chloride concentration of 0.25% in the reagent.

Effect of Quality of Triethanolamine. Figure 2 shows calibration curves obtained for ethanolamine using a highly purified grade of triethanolamine (curve 1) and commercial triethanolamine (curve 3), and demonstrates that the quality of triethanolamine used in the reagent determines the slope of the calibration curve. This is not desirable because a separate calibration curve would be required for each lot of triethanolamine used in the reagent. This effect is no doubt caused by the presence of chelating agents in commercial triethanolamine which effectively decrease the cupric ion concentration of the reagent, thus decreasing the sensitivity of the method as shown by curve 1 in Figure 1. To establish the theoretical calibration curve for a primary amine, a calibration curve (curve 2, Figure 2) was obtained for copper acetate, employing the relative volumes used in the amine procedure. For these curves a plot of absorbance *vs.* microequivalents was made. Within the experimental error of the determinations, curves 1 and 2 are identical. Therefore, a refined grade of triethanolamine is necessary for the preparation of the reagent if a reproducible calibration curve is desired. The method of preparation of a suitable grade of triethanolamine is described below.

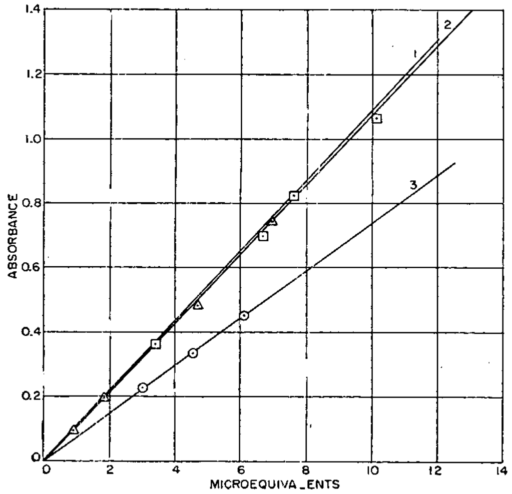

Figure 2. Effect of triethanolamine quality on slope of calibration curve for ethanolamine

1. Ethanolamine, using refined triethanolamine in reagent ⊡
2. Cupric acetate monohydrate ◬
3. Ethanolamine, using commercial triethanolamine in reagent ⊙

Table I. Reaction Conditions for Determination of Primary Amines by Copper-Salicylaldehyde Method

Compound	Primary Amine, Mg., Maximum	Time, Minutes[a]
Aminoethylethanolamine	1.10	30 to 60
N-Aminoethylmorpholine	0.85	15 to 60
Amylamine	1.20	15 to 45
Butylamine	0.70	15 to 60
Ethanolamine	0.50	15 to 60
Ethylamine	0.53	15 to 60
2-Ethylhexylamine	1.40[b]	15 to 60
Hexylamine	1.10	15 to 60
Isoamylamine	1.10	15 to 45
Isobutanolamine	0.60	60 to 120
Isobutylamine	0.90	15 to 60
Isopropanolamine	0.60	15 to 60
Methylamine	0.30	15 to 60
Propylamine	0.73	15 to 60
Propylenediamine	0.42	10 to 20[c]

[a] Reaction time at 20° to 30° C. unless otherwise specified.
[b] Make dilutions using a 10% solution of methanol.
[c] Perform reaction at 98° ± 2° C. Use 50-ml. glass-stoppered graduated cylinders; do not stopper during reaction.

METHOD

Reagents. Cupric chloride dihydrate.

1-Hexanol, Carbide and Carbon Chemicals Co.

Salicylaldehyde, reagent grade.

TRIETHANOLAMINE, Carbide and Carbon Chemicals Co. Distill 98% material under 1- to 2-mm. pressure, using a column 6 inches long and 30 mm. in diameter packed with 2-mm. glass beads and heated by means of resistance wire. Use a 3-liter, round-bottomed distillation flask fitted with a thermometer well. Stir the contents of the flask by means of a magnetic stirrer, and do not allow the kettle temperature to exceed 185° C. during the distillation. An absorbance of 0.65 ± 0.02 for 0.372 mg. of ethanolamine should be obtained by the procedure described below, when this material is used to prepare the copper-salicylaldehyde reagent.

COPPER-SALICYLALDEHYDE REAGENT. Into a 100-ml. glass-stoppered graduated cylinder measure 15.0 ml. of redistilled triethanolamine, 0.5 ml. of salicylaldehyde, and 0.25 gram of cupric chloride dihydrate. Dilute to 100 ml. with distilled water and mix the contents. This reagent is stable for at least a month; however, the reagent blank increases with age.

BIS(2-HYDROXYETHYL)DITHIOCARBAMIC ACID REAGENT. Prepare a 2% by volume solution of carbon disulfide in methanol and a 5% by volume solution of diethanolamine in methanol. Prepare the reagent fresh daily by mixing equal volumes of the two components.

Calibration Curve. Prepare a dilution of the pure compound in distilled water so that a 5-ml. aliquot contains not more than the maximum sample size given in Table I. To each of five 25-ml. glass-stoppered graduated cylinders add 2 ml. of copper-salicylaldehyde reagent by means of a pipet. Transfer 1.0-, 2.0-, 3.0-, and 5.0-ml. aliquots of the above dilution to respective 25-ml. graduated cylinders, reserving one as the blank. Measure the absorbance of each standard at 430 mμ, using 1-cm. cells and a suitable spectrophotometer.

Procedure. Add 2.0 ml. of copper-salicylaldehyde reagent from a transfer pipet to each of two 25-ml. glass-stoppered graduated cylinders. Reserve one of the cylinders as a blank, and into the other measure an amount of sample calculated to contain not more than the maximum amount of primary amine listed in Table I. The sample must not contain more than 0.01 mg. of ammonia or 0.5 gram of secondary and tertiary amine. For samples of less than 0.1 gram use an aliquot of a suitable aqueous dilution. Dilute the contents of each graduate to the 10-ml. mark with distilled water, stopper, and mix thoroughly. Allow the sample to react under the conditions specified in Table I.

After the reaction is complete add sufficient 1-hexanol to bring the total volume of liquid to 25 ml. Stopper the cylinders, shake vigorously 15 or 20 times, and allow the layers to separate. Add 5 ml. of bis(2-hydroxyethyl)dithiocarbamic acid reagent to each of two additional 25-ml. glass-stoppered graduated cylinders. In this step it is important that the graduated cylinders and stoppers be clean and void of any metallic ions that react with this reagent. Pipet 5.0 ml. of the hexanol layer from the graduated cylinders in which the reaction was performed to the graduates containing the dithiocarbamic acid reagent. Add the hexanol dropwise to prevent the material from clinging to the walls of the pipet. Dilute the contents of each cylinder to the 25-ml. mark with methanol, stopper, and mix the contents. Measure the absorbance of the sample *vs.* the blank at 430 mμ, using 1-cm. cells. Read the concentration of primary amine from the calibration curve.

DISCUSSION AND RESULTS

A reaction rate study and a calibration curve were obtained for each primary amine investigated. Listed in Table I are the

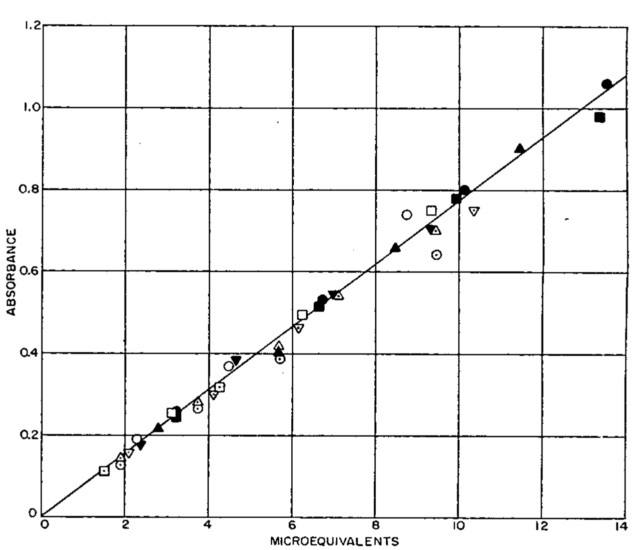

Figure 3. Calibration curve for 10 primary amines

⊙ *n*-Amylamine	■ Ethylamine
⊡ Isoamylamine	▲ *n*-Hexylamine
◬ Isobutylamine	▼ 2-Ethylhexylamine
▽ *n*-Propylamine	□ Aminoethylethanolamine
● Propylenediamine	○ *n*-Butylamine

Table II. Determination of Primary Amines in Presence of Secondary and Tertiary Amines

Sample	Primary Amine, % by Weight		
	Added	Found[a]	Diff.
Isopropanolamine in 2,5-dimethylpyrazine	0.12	0.12 (2)	0.00
	0.57	0.57 (2)	0.00
Ethanolamine in diethanolamine	2.35	2.16 (1)	−0.19
	0.36	0.32 (2)	−0.05
	0.12	0.13 (2)	+0.01
	0.09	0.13 (2)	+0.07
	2.4	2.5 (1)	+0.1
Ethanolamine in triethanolamine	0.31	0.31 (1)	0.00
	0.10	0.10 (1)	0.00
	0.98	0.90 (1)	−0.08
Ethanolamine in di- and triethanolamine	47.6	47.6 (2)	0.0
	21.3	21.2 (2)	+0.1
	29.2	28.7 (4)	−0.5
Butylamine in dibutylamine	1.07	1.14 (1)	+0.07
	0.56	0.53 (1)	−0.03
	0.22	0.15 (1)	−0.07
	0.49	0.54 (1)	+0.05

[a] Figures in parentheses represent number of determinations.

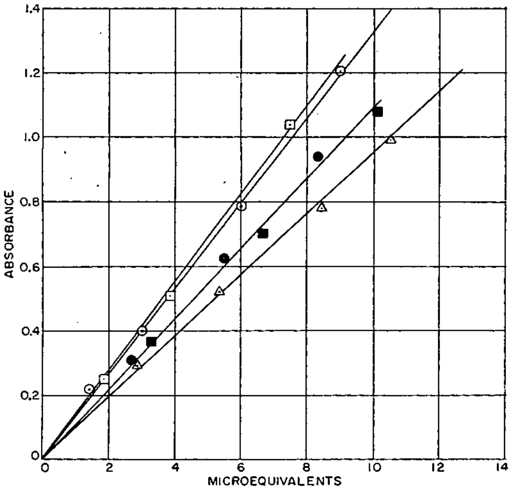

Figure 4. Calibration curves for primary amines that deviate from curve in Figure 3

⊙ Isobutanolamine
△ Methylamine
⊡ *N*-Aminoethylmorpholine
● Isopropanolamine
■ Ethanolamine

recommended reaction conditions for primary amines to which this method has been applied. In all cases the maximum sample size is based on the amount of pure primary amine corresponding to an absorbance of 0.9 under the conditions of the method. Figure 3 shows the calibration curves obtained for 10 primary amines. For this curve absorbance is plotted *vs.* microequivalents of primary amine. Using such a plot all primary amines should fall on the same calibration curve, provided: (*a*) the ratio of amine to copper is constant; (*b*) a quantitative extraction of the complex from water is obtained; or (*c*) the distribution coefficient is the same for each compound. Figure 3 shows that, within certain limits, for these 10 primary amines one calibration curve suffices. Of all the compounds investigated five primary amines did not fall on this curve. Figure 4 shows the calibration curves obtained for these compounds, three of which are alcoholamines. The sensitivity is greater for these compounds than for the other amines investigated. The calibration curves obtained for ethanolamine and isopropanolamine correspond to the theoretical curve for a 1 to 1 mole ratio of copper to amine as shown in Figure 2. This is not in agreement with the findings of other investigators who report a 2 to 1 ratio of amine to copper (*5*). Evidently in dilute solution the formation of the 1 to 1 complex is favored. In no case was a calibration curve obtained that corresponded to the 2 to 1 complex.

A few primary amines do not react quantitatively with this reagent. In general, compounds that react incompletely can be divided into three classes: (*a*) aromatic amines such as aniline; (*b*) compounds that contain more than one primary amine group—e.g., ethylenediamine and diethylenetriamine; and (*c*) primary amines that are branched in the 2 position, such as tertiary and secondary butylamine and isopropylamine. Propylenediamine and 2-ethylhexylamine are exceptions to this generalization.

Ammonia interferes if more than 0.01 mg. is present in the sample aliquot. This amount of ammonia corresponds to an absorbance of 0.03, which is within the experimental error of the method. Attempts to determine ammonia by this method were unsuccessful, because the reaction apparently is not quantitative. The combined secondary and tertiary amine content of the sample aliquot must not exceed 0.5 gram. Greater amounts of secondary or tertiary amines tend to solubilize the copper complex in the aqueous layer. Strong oxidizing or reducing agents interfere by depleting the reagent. Compounds that form copper complexes soluble in 1-hexanol give high results; those forming water-soluble complexes give low results.

This method has been successfully applied to the analysis of several amine mixtures. To date the method has replaced the Van Slyke method in many cases for the control of plant processes with a considerable saving of time and laboratory costs. In Tables II, III, and IV are listed data for the analysis of several mixtures of primary, secondary, and tertiary amines. Table II shows the determination of several amine mixtures of known primary amine content. In Tables III and IV, analyses of several plant process samples are given. In each case, the primary amine content was determined by the copper-salicylaldehyde and Van Slyke methods. The data in Table III were obtained by an experienced operator, while those in Table IV were obtained in a routine control laboratory. For the determination of primary amines in the presence of secondary and tertiary amines an accuracy within 5% can be anticipated by this method. In some cases Van Slyke and copper-salicylaldehyde comparative results are substantially better (Table IV), while in others they are

Table III. Determination of Ethanolamine in Presence of Di- and Triethanolamines

Sample No.	Ethanolamine, % by Weight		
	Van Slyke method	Copper-salicylaldehyde method	Diff.
1	11.5	12.1	+0.6
2	12.9	13.0	+0.1
3	12.5	12.5	0.0
4	11.7	12.2	+0.5
5	13.1	12.5	−0.6
6	10.8	11.1	+0.3
7	13.1	12.1	−1.0
		Av. diff.	±0.5

Table IV. Control Laboratory Determination of Ethanolamine in Presence of Di- and Triethanolamine

Sample	Operator	Ethanolamine, % by Weight		
		Van Slyke method	Copper-salicylaldehyde method	Diff.
1	1	42.2	41.8	−0.4
2	1	39.1	41.2	+2.1
2	2	39.5	40.1	+0.6
3	1	38.3	36.6	−1.7
3	2	42.2	42.4	+0.2
4	1	34.3	35.4	+1.1
4	2	36.4	37.2	+0.8
5	2	14.4	14.5	+0.1
			Av. diff.	±0.9

somewhat poorer. Because of the limited sample size, primary amine contents of less than 0.01% in the presence of secondary and tertiary amines cannot be determined by this method. The method was successfully applied to the determination of ethanolamine hydrochloride without prior neutralization of the sample. The absorbance of the hydrochloride is proportionally the same as the free amine.

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Received for review October 21, 1955. Accepted February 7, 1956.

Coulometric Titration of Ammonia with Hypobromite

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A coulometric method has been developed for the titration with electrolytically generated bromine of microgram quantities of ammonia in a solution having a pH of 8.5. The effects of pH and of certain metal ions on the titration have been investigated. Quantities of ammonia ranging from 14 to 230 γ have been determined with an average error of less than ± 0.2 γ.

WILLARD and Cake (*14*) and Kolthoff and Stenger (*7*) have developed volumetric methods for the determination of ammonia by oxidation with hypobromite according to the following equation:

$$2NH_3 + 3BrO^- = 3Br^- + N_2 + 3H_2O \qquad (1)$$

More recently, amperometric end points have been described for titrations which are based on the same reaction (*8*, *9*).

Bromine disproportionates in basic solutions (*6*) according to the equation

$$Br_2 + 2OH^- = BrO^- + Br^- + H_2O \qquad (2)$$

Thus, it seemed that a coulometric determination of ammonia might be developed which utilized the electrolytic generation of bromine and was based on the reactions represented by Equations 1 and 2. The possibility that such a method could be used for the direct determination of the ammonia in a Kjeldahl digestion solution justified the investigation described.

EXPERIMENTAL

Reagents. All solutions were prepared from reagent grade chemicals. Water was distilled from alkaline potassium permanganate in order to remove most of the reducing materials normally found in the tap distilled water. This special water was prepared in lots of about 2 liters each. Buffer solutions were prepared by dissolving 4.8 grams of sodium tetraborate decahydrate in 500 ml. of water to give solutions which were 0.025VF (volume formal) in sodium tetraborate. The pH was adjusted to the desired value by adding sodium hydroxide pellets or concentrated perchloric acid as required.

Standard solutions of ammonium chloride from 0.033 to 0.074VF were prepared by dissolving weighed quantities of dried salt (Baker's analyzed reagent) in the specially distilled water in a volumetric flask. Aliquots of these standard solutions were diluted to give concentrations ranging from $8.34 \times 10^{-5}VF$ to $1.34 \times 10^{-3}VF$.

Bromine solutions were prepared by adding 5 grams of sodium bromide and 3 ml. of saturated bromine water to 1 liter of distilled water. These solutions were standardized by coulometric titration with cuprous copper as described below.

Apparatus. All volumetric equipment was calibrated.

The titration apparatus was similar to that described by Meier, Myers, and Swift (*10*) as modified by Ramsey, Farrington, and Swift (*12*). New equipment was constructed which had the following changes.

The current regulating vacuum tubes, Type 1C5, were replaced by Type 6AU6, because the latter have more stable operating characteristics and give more dependable service. The 1$^1/_2$-volt dry cell which supplied the filament voltage for the vacuum tubes was replaced by a 6-volt storage battery (Willard Signal Corps Battery BB-207/U); and the 1$^1/_2$-volt dry cell which supplied the indicator potential was replaced by a 2-volt storage cell (Willard Signal Corps Battery BB-54-A).

The rubber stopper which served as a cap for the titration cell during operation was equipped with a short piece of glass tubing flared at the top to allow addition of solutions to the cell without exposing the electrodes to the air.

The indicator electrodes were made of pieces of sheet platinum 2 cm. by 2.5 cm.

A Leeds & Northrup box-type reflecting galvanometer, shunted with a rheostat to provide variable sensitivity, was used for indicator current readings near the end point.

The generating current was determined by measuring the voltage drop across a Leeds & Northrup standard mercury resistor (99.99_8 absolute ohms) through which the current was passed. This current corresponded to $4.078_2 \times 10^{-8}$ equivalent per second at the medium rate and to $1.004_5 \times 10^{-7}$ equivalent per second at the high rate.

The voltage applied across the two indicator electrodes (the "indicator potential") was measured with a Gray Instrument Co. Queen potentiometer.

The solution in the isolated electrode compartment was 0.3VF perchloric acid.

End Point Methods. Experiments showed that the indicator current resulting from a given quantity of bromine in an alkaline solution was not sufficiently reproducible for use as a means of determining the end point. Therefore the current in the alkaline solution was used only to indicate when a slight excess of bromine had been generated. Two methods for determining this excess were then considered.

In the first and recommended method the solution is acidified and bromine again generated until a predetermined current value is obtained. In the second method the solution is acidified, cupric copper added, and the excess bromine back-titrated with cuprous copper as described by Buck and Swift (*2*).

Selection of Indicator Potential. An indicator potential of 150 mv. was used in both methods of determining the end point. In the first method described above this value was found to be sufficiently high to give an indication of the presence of excess bromine in the alkaline solution without causing an excessive current when the solution was acidified.

Farrington, Meier, and Swift (*5*) found that the optimum applied indicator potential for the determination of the minimum indicator current in their cupric copper–bromide system was 60 mv. Fluctuations of indicator potential should then have a minimal effect on the minimum indicator current. On the other hand, Buck and Swift (*2*) used an indicator potential of 200 mv. for a simple system in which an arbitrary current caused by an excess of bromine was used. However, the bromide concentrations used in the experiments reported here are considerably higher than those used by Buck and Swift and caused higher minimum currents. In order to avoid high minimum currents, a low indicator potential is desirable. Experiments showed that the indicator current was no longer a linear function of concentration after 4 or 5 seconds of generation when the indicator potential was as low as 90 mv., but that linearity was maintained over the desired range when the indicator potential was greater than 120 mv. Most experiments were performed with an indicator potential of 150 mv.

All pH measurements were made with a Beckman Model F pH meter.

Generator Current Efficiency in Alkaline Solutions. That hypobromite could be produced with 100% current efficiency in alkaline solutions was demonstrated by generating bromine for a known time under the conditions of a titration, then acidifying the solution, adding cupric copper, reversing the polarity, and titrating the bromine by generation of cuprous copper. The second end point method described above was used, and an arbitrary current value was taken as the end point of the latter titration. An end point correction was made as follows. To 25.0 ml. of water, 15.0 ml. of buffer (pH 9.3), and 10.0 ml. of 5*VF* sodium bromide were added 2.0 ml. of 9*VF* perchloric acid and 1.0 ml. of 1*VF* copper sulfate. The resulting acid concentration was 0.3*VF*. Bromine was generated in 1.0 second intervals and the stable indicator current recorded after each generation. The indicator current was considered to be stable when it did not vary by more than 0.2 to 0.3 μa. in 30 seconds. A straight line was passed through the plotted points and a predetermined arbitrary current value was selected as the end point current. The time corresponding to this current was designated as the end point correction time.

Then, to check the generator current efficiency, a solution was prepared which contained 25.0 ml. of water, 15.0 ml. of buffer (pH 9.3), and 10.0 ml. of 5*VF* sodium bromide; this solution had a final pH of 8.8. In all cases, the pH of the prepared titration solution was 0.3 to 0.5 unit less than that of the buffer. The ionic strength of the buffer was about 0.05*VM*, and that of the titration solution was about 1*VM*. Bromine was generated for 30.0 seconds, whereupon 2.0 ml. of 9*VF* perchloric acid and 1.0 ml. of copper sulfate were added, bringing the acid concentration to 0.3*VF*. The polarity of the generator electrodes was reversed and cuprous copper was generated until the indicator current decreased to about 40 μa. The indicator current was recorded when it became stable. Generation of cuprous copper was continued in 1.0-second intervals until the indicator current had decreased below the arbitrary end point value. A straight line was drawn through the plotted points and the time corresponding to the end point current was taken as the titration time. The end point correction time was subtracted from the titration time to yield a value from which to calculate the equivalents of cuprous copper generated. In general, the number of equivalents was not calculated, but comparison was made by considering the anodic and cathodic generation times. As the result of such checks, it was found in every case that less cuprous copper than bromine was generated. The apparent loss of bromine was equivalent to about 7 to 8 seconds of generation, and was reproducible to within 0.2 second. Therefore, two sets of experiments were performed to attempt to determine if the error was due to low generation efficiency or if bromine was being lost in some manner.

End point correction determinations were made as previously described. Titration solutions were prepared by taking 15.0 ml. of water, 15.0 ml. of buffer solution, 10.0 ml. of 5*VF* sodium bromide, and 10.00 ml. of standard bromine solution. The pH of the solutions was 8.8. The solutions were allowed to stand for about 30 seconds, then 2.0 ml. of 9*VF* perchloric acid and 1.0 ml. of 1*VF* copper sulfate were added and the bromine was titrated as described previously. Again, the losses of bromine were equivalent to about 7 seconds of generation. Similar experiments were performed in which bromine was added to the cell when the platinum electrodes were present to see if the electrodes had a catalytic effect; also the quantity of bromine added was varied to see if the effect was dependent on bromine concentration. In all cases the loss was the same, suggesting that impurities in the solution were being oxidized by hypobromite and that bromine could be generated in alkaline solution with 100% current efficiency. Because the losses were reproducible, the decision was made to use a combined reagent and end point correction procedure rather than to attempt to purify all the reagents.

PROCEDURE

Reagent and End Point Correction. The blank solution contained 25.0 ml. of water, 15.0 ml. of buffer solution (pH 8.9), and 10.0 ml. of 5*VF* sodium bromide. The pH of the solution was 8.5. Bromine was generated for 8 to 10 seconds and the indicator current allowed to stabilize. It was found that if the current was less than 10 μa., the reducing impurities were not completely removed. When the current stabilized at about 10 μa., 2.0 ml. of 9*VF* perchloric acid were added, causing a rise in the indicator current to about 25 μa. The current was recorded and bromine was generated in 1.0-second intervals, the stable current being recorded at each interval. A straight line was drawn through the plotted points and the time corresponding to 30 μa. was taken as the reagent and end point correction time. Correction determinations were repeated until three consecutive determinations agreed within 0.2 second.

Titration. The titration solution contained 15.0 ml. of water, 15.0 ml. of buffer solution (pH 8.9), 10.0 ml. of 5*VF* sodium bromide, and 10.00 ml. of standard ammonium chloride solution. The ammonium chloride solution was added just before the cell was mounted, so that losses through volatilization might be reduced. As bromine was generated, the indicator current usually rose to about 20 μa. and leveled off at about that value for most of the titration. Near the end of the titration, the current began to rise and the titration was stopped when the rate of rise was about 1 μa. in 5 seconds (usually 5 to 15 seconds before the end point).

The current was allowed to decrease until stable and, if it was lower than 10 μa., generation was continued in short intervals until a stable current of about 10 μa. was obtained. Then 2.0 ml. of 9*VF* perchloric acid were added and the generation of bromine was continued as described for the reagent and end point correction. The reagent and end point correction time was subtracted from the titration time to obtain a corrected time; this time was then used to calculate the quantity of ammonia titrated.

DISCUSSION AND RESULTS

Effect of pH. Previous workers (*8*) have found that the accuracy of the volumetric titration of ammonia with hypobromite is critically dependent upon the pH of the solution. Among the factors which are involved is the stoichiometry of the titration. Clusius and Rechnitz (*4*) and Riley and others (*13*) have shown that under certain conditions significant quantities of nitrous oxide may be formed by the following reaction:

$$2NH_3 + 4BrO^- = N_2O + 4Br^- + 3H_2O \quad (3)$$

The stoichiometry of the titration is also affected by the extent to which the reaction

$$3\ BrO^- = BrO_3^- + 2Br^- \quad (4)$$

takes place, since bromate ion does not oxidize ammonia. Finally, the possibility of volatilization of ammonia from the solution becomes greater with increase in pH.

Because of these considerations a series of experiments was made in order to establish the permissible pH limits for the coulometric titration.

The end point correction and titration procedures used were as described above, with the exception that the pH of the system was varied. The results of a series of experiments in which the pH was varied between 8.2 and 10.0 are shown in Table I. It can be seen that 8.5 and 8.2 are the best pH values of those

Table I. Effect of pH on Determination of Ammonia

No. of Detn.	pH	Ammonia, γ Taken	Ammonia, γ Found	Error	Standard Dev., γ
3	10.0	56.81	55.72	−1.1	±0.14
7	8.8	227.4	226.2	−1.2	±0.3
5		56.81	56.62	−0.19	±0.12
5	8.5	227.5	227.3	−0.2	±0.2
6		56.81	56.98	+0.17	±0.12
1	8.2	227.4	227.7	+0.3	
3		56.81	57.17	+0.36	±0.20

Table II. Effect of Excess Hypobromite on Determination of Ammonia

(Ammonia sample was added to the solution after a quantity of bromine had been generated. Equivalents of bromine initially generated are shown in second column; total generated for titration are shown in third column. pH of each solution was 8.8.)

No. of Detn.	Bromine Generated, Equiv. × 10^7 Initial	Bromine Generated, Equiv. × 10^7 Total	Ammonia, γ Taken	Ammonia, γ Found	Error	Std. Dev., γ
1	230	401	227.4	228.8	+1.4	
4	200	401	227.4	228.0	+0.6	±0.2
3	100	401	227.4	227.5	+0.1	±0.2
7	0	401	227.4	226.2	−1.2	±0.3

tried. However, a system at a pH of 8.2 is not convenient, because attainment of constant indicator currents at this pH value was excessively slow. Kolthoff and others (*7*) found that a pH of 8.5 was most satisfactory for their procedure.

It is seen that the observed errors shift from negative to positive as the pH of the system decreases. Although the reason for this is not definitely known, it is possible that at the higher pH values loss of ammonia may be the predominant source of error, while formation of nitrous oxides may predominate at the lower values. It is also possible that compensation of these errors may give the best results at pH 8.5.

Other buffer systems were considered for the control of the pH during the titration. The carbonate-bicarbonate system is impractical for the method used here because the formation of carbon dioxide on acidification of the solution would tend to sweep bromine from the solution. Phosphate buffers were not used because the available reagents apparently contained ammonia or other reducing impurities, which would have caused the blank corrections to be unreasonably large.

Effect of Excess Hypobromite. The fact that fairly large negative errors were found when 227 γ of ammonia were titrated at pH 8.8 suggested the possibility of adding the sample after some quantity of bromine had been generated, thus allowing less time for the ammonia to escape from the solution. A series of experiments was performed in which bromine was generated for certain fixed times, after which the sample was added and the titration continued. The results are shown in Table II. It can be seen that the positive error increased as the initial excess of bromine (or hypobromite) was increased. This may be due to an increased formation of nitrous oxide when the hypobromite is in excess.

End Point Method. The optional end point methods have been described. An end point in the alkaline solution would have eliminated the necessity for adding acid to the solution with the resulting high indicator current. However, it was found that the indicator current in alkaline solution was not reproducible and did not give a reliable measure of the amount of bromine which was present.

Another reason for determining the end point in acid solution is that it allows the possibility of back-titration with cuprous copper if the end point is badly overrun. However, if this back-titration method is used, care must be exercised to use exactly the same technique for the blank as for the titration. A few runs were made in which the blank correction was determined as described above and the titration was accomplished by a back-titration method. In these cases, the errors were as large as +0.6 γ, compared with an error of 0.2 γ when the end point was determined as described in the procedure. The slope of the indicator current–time curve was greater when the bromine concentration was decreased by generating cuprous copper than when the bromine concentration was increased by generating bromine. The reason for this difference is not known. However, it was sufficient to cause observed differences in the accuracy of the titration.

The concentration of sodium bromide used (approximately 1*VF*) was that which experiments had shown to be satisfactory for the determination of bromate by addition of bromide and cupric copper to an acid solution and titration by generation of cuprous copper. These experiments were made to provide for the possibility that significant quantities of bromate would be formed during the titration of ammonia with bromine in an alkaline solution. Buck and Swift (*2*) used a bromide concentration of 0.1*VF* for the determination of aniline; therefore, a comparable concentration may be practical for the determination of ammonia.

Experiments in connection with the bromate determination had shown that the lowest practical acid concentration was 0.3*VF*.

The procedure used by Laitinen (*9*), in which a single indicator electrode is employed, has the advantage that the end point can be determined in alkaline solution. However, if small quantities of bromate are formed, positive errors result, because it was found that the bromate does not oxidize ammonia under the conditions of the titration.

Effect of Other Metal Ions. If the procedure described above could be utilized to eliminate the distillation process in the micro-Kjeldahl determination of nitrogen, it would greatly increase the ease with which that procedure is carried out. Therefore, experiments were made to determine if the materials commonly employed as catalysts in the Kjeldahl digestion would interfere with the coulometric determination of ammonia. The procedure just described was used, except that amounts of copper sulfate, mercuric nitrate, and selenium were added which were typical of the amounts which would be found in a micro-Kjeldahl determination (*3*, *11*).

Table III. Effect of Metals on Determination of Ammonia

[Concentration of $CuSO_4$ was 6 × 10^{-4}*VF*, and of $Hg(NO_3)_2$, 2 × 10^{-4}*VF*.]

Added Metal	Ammonia, γ Taken	Ammonia, γ Found	Error
Cu(II)	227.5	227.8	+0.3
		227.2	−0.3
		Av. 227.5	+0.0
	56.81	57.13	+0.3
		57.06	+0.2
		56.82	0.0
		Av. 57.00	+0.2
Hg(II)	227.5	227.1	−0.4
	56.81	56.64	−0.2
		56.93	+0.1
		56.66	−0.2
		Av. 56.74	−0.1

Selenium metal was dissolved in hot concentrated sulfuric acid, then slowly diluted with water, and the solution was neutralized to a pH of 4. On dilution, some selenium reprecipitated. A portion of the mixture was filtered and experiments were performed with small quantities of the mixture and of the filtrate.

The results with copper sulfate and mercuric nitrate are shown in Table III. It can be seen that neither copper sulfate nor mercuric nitrate affects the accuracy of the ammonia determination.

The presence of selenium affects the titration, however. No actual titration was made with selenium compounds present, because it was found that the selenium in solution reduced hypobromite as rapidly as it was formed. Although the species of selenium involved is not known, experiments made with selenious acid showed that selenite reduced hypobromite. It is evident that if selenium is to be used as a catalyst, some way must be found to eliminate interference before the ammonia determination is begun. Experiments were performed in which metallic lead was added to the sulfuric acid solution containing selenium in the hope that the selenium would be reduced to the metal. Although most of the selenium was removed from the solution in this way, 4 days were required to complete the reduction. Reduction with sulfur dioxide (*1*) was not considered because of the time required for removal of the excess.

Table IV. Confirmatory Titrations

No. of Detn.	Ammonia, γ Taken	Found	Error	Std. Dev., γ
5	227.5	227.3	−0.2	±0.2
6	125.8	125.9	+0.1	±0.2
6	56.81	56.98	+0.17	±0.12
3	14.20	14.20	0.00	±0.22

Confirmatory Titrations. Table IV shows the results of a series of confirmatory titrations made by the procedure described above. In all cases, the average error was less than 0.2 γ, while the standard deviation was about the same. Thus, one might expect a maximum error of 0.4 γ in a determination involving quantities of ammonia between 14 γ and 230 γ. Both the relative and absolute accuracies are somewhat better than those reported by Laitinen (*9*).

ACKNOWLEDGMENT

Preliminary experiments made by Fred C. Anson have been helpful in this work. G. Myron Arcand is indebted to the General Electric Co. for a predoctoral fellowship during the academic year 1954–55.

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Received for review July 18, 1955. Accepted January 16, 1956. Contribution No. 2016 from the Gates and Crellin Laboratories of Chemistry.

Coulometric Titrations with Electrically Released Ethylenediaminetetraacetic Acid

Titrations of Calcium, Copper, Zinc, and Lead

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Successful coulometric titration of calcium, copper, zinc, and lead ions was accomplished by the indirect electrical generation of ethylenediaminetetraacetic acid released upon reduction of mercuric–ethylenediamine tetraacetate chelate at a mercury pool. The pertinent equilibrium conditions are summarized in a potential-pH diagram. This diagram, in conjunction with polarograms for kinetic effects, furnished valuable information concerning the solution conditions desired. Extension of this method to the titration of many other metal ions appears feasible.

Coulometric titrations with constant current have been employed in various types of oxidation-reduction, precipitation, and neutralization reactions. The present investigation was undertaken to test the feasibility of extending the application of coulometric titrations into compleximetry. If a general method for the coulometric generation of ethylenediaminetetraacetic acid [EDTA, H_4Y, (ethylenedinitrilo)tetraacetic acid] could be found, it would be highly probable that the coulometric method could be used for the successful titration of the alkaline earths, the rare earths, and such metal ions as manganese(II), iron(II), copper(II), mercury(II), cobalt, nickel, zinc, cadmium, lead, aluminum, gallium, indium, thorium, palladium(II), and bismuth. The solution of this problem required the discovery of a mode of generating ethylenediaminetetraacetic acid with 100% current efficiency, and the development of a compatible and sensitive end-point detection device. The properties of the end-point method selected are discussed in greater detail elsewhere (*8*).

Mode of Generation. Several modes of coulometric titration of metal ions through the formation of stable chelates with ethylenediaminetetraacetic acid suggest themselves.

The formation of the reagent, ethylenediaminetetraacetic acid, through an electrode process such as the oxidation of ethylenediaminetetraacetaldehyde, was ruled out on the basis of requiring reagents which were not commonly available in pure form and because doubt existed about the successful generation at 100% current efficiency of ethylenediaminetetraacetic acid in such a manner.

It seemed that either of the following two indirect titration procedures might be employed.

The first would require the coulometric generation of base in order to titrate the acid liberated when an excess of ethylenediamine tetraacetate (at a pH of either 4.5 or 8.2) is added to the solution of the metal ion at a corresponding pH. For example:

$$\overset{\text{pH} = 8.2}{HY^{-3}} + \overset{\text{pH} = 8.2}{Ca^{++}} \rightarrow CaY^{--} + H^+ \qquad (1)$$

The H^+ would then be titrated, until the pH returns to 8.2, by the OH^- formed by the following electrode reaction:

$$2H_2O + 2e \rightarrow H_2\uparrow + 2OH^- \qquad (2)$$

The simultaneous electrolytic reduction of any divalent metal ion would lead to high results and must be prevented. This procedure is also undesirable because of the necessity of having the pH of the sample solution and the ethylenediaminetetraacetic acid solution carefully controlled at 4.5 or preferably 8.2 prior to the titration.

The second indirect titration procedure would function in the following manner.

A known amount of standard ethylenediaminetetraacetic acid is added in excess to the sample containing the metal ion. The excess ethylenediaminetetracetic acid is then back-titrated by a metal ion derived from the anodic dissolution of a metal.

$$HY^{-3} + Ca^{++} \rightarrow CaY^{--} + H^+ \qquad (3)$$

$$M \rightarrow M^{+n} + ne \qquad (4)$$

$$M^{+n} + HY^{-3}\,(\text{excess}) \rightarrow MY^{n-4} + H^+ \qquad (5)$$

Although this method permits the use of buffered solutions of pH's where selected reactions might occur, it does not eliminate the preparation and use of a standard ethylenediamine tetraacetate solution.

The method finally evolved consisted of the liberation of ethylenediaminetetraacetic acid by the following electrode process:

$$HgY^{--} + 2e \rightarrow Hg^0 + Y^{-4} \qquad (6)$$

The ethylenediamine tetraacetate thus liberated is used to titrate the metal ion of the sample:

$$Ca^{++} + Y^{-4} \rightarrow CaY^{--} \qquad (7)$$

In this procedure, the simultaneous reduction of divalent ions to metals does not cause any error. The selection of mercury for the metal–ethylenediamine tetraacetate complex was based on the following principles.

First, it was desirable that the stability constant of the complex be large. The metal–ethylenediamine tetraacetate complex would then have sufficiently great stability to allow titrations in acid solutions. It was also desirable that the hydroxide (or oxide) of the metal selected have a sufficiently large solubility product relative to the instability constant of the metal–ethylenediamine tetraacetate complex, so that the complex would not decompose into insoluble hydroxides (or oxides) in alkaline solutions. Mercury–ethylenediamine tetraacetate complex, with an instability constant of approximately 10^{-22}, seemed to be a favorable choice.

A second factor concerns the interference of oxygen through simultaneous reduction in the electrode generation process. Polarograms show that mercury has the most positive reversible half-wave potential of the common metal ions that also possess sufficient stability with ethylenediamine tetraacetate. Even in the case of mercury it was found necessary to exclude oxygen from the electrolysis cell. Employment of metals with more negative half-wave potentials would require an even more careful elimination of oxygen.

Selection of a metal with a high positive half-wave potential is also desirable in minimizing the extent of the undesirable side reaction, the liberation of hydrogen. For example, it would be even more difficult to attain 100% current efficiency if zinc–ethylenediamine tetraacetate complex were used. In fact, Pecsok (*6*) reports that no reduction wave was obtained at the dropping mercury electrode for lead, cadmium, or zinc chelates. The large hydrogen overvoltage on mercury is also desirable.

Other factors which influenced the choice of mercury–ethylenediamine tetraacetate complex were the ease of using a mercury pool as the generating electrode and the mercury drop as a corresponding end-point detection device. Back-titration was also possible by simply reversing the direction of current flow. A black substance (probably mercurous oxide) was sometimes obtained near the electrode surface with anodic currents. It, however, quickly disappeared upon stirring and had no noticeable effect on the results.

POTENTIAL-pH DIAGRAM

The characterization of the selected chemical system at equilibrium is summarized by the potential-pH diagram shown in Figure 1. This diagram may be used to predict the effectiveness of the coulometric titration under various pH conditions and for metal–ethylenediamine tetraacetate chelates of differing stability. In addition the properties of the potentiometric end point under these same conditions can be elucidated.

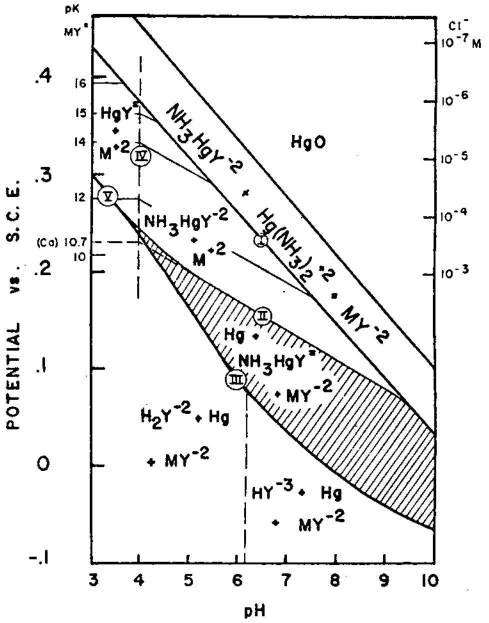

Figure 1. Potential-pH diagram

The diagram was constructed from a combination of experimental data and reported equilibrium constants (*3*, *5*, *9*). The equilibrium constant for Equation 14 was calculated from experimental data. The following equilibria are pertinent:

$$NH_4^+ \rightarrow H^+ + NH_3 \quad pK = 4.7 \qquad (8)$$

$$H_nY^{n-4} \rightarrow H^+ + H_{n-1}Y^{n-5}$$
$$pK_1 = 10.26 \quad pK_2 = 6.16 \quad pK_3 = 2.76 \qquad (9)$$
$$pK_4 = 2.0 \text{ where } n = 1, 2, 3, 4 \text{ respectively}$$

$$HgY^{--} \rightarrow Hg^{++} + Y^{-4} \quad pK = 22.1 \qquad (10)$$

$$CaY^{--} \rightarrow Ca^{++} + Y^{-4} \quad pK = 10.7 \qquad (11)$$

$$MY^{--} \rightarrow M^{++} + Y^{-4} \quad pK = 10, 12, 14, 15, 16, \ldots \qquad (12)$$

$$Hg(NH_3)_2^{++} \rightarrow Hg^{++} + 2NH_3 \quad pK_1K_2 = 17.5 \qquad (13)$$

$$NH_3HgY^{--} \rightarrow HgY^{--} + NH_3 \quad pK = 6.6 \qquad (14)$$

$$HgO(r) + H_2O \rightarrow 2OH^- + Hg^{++} \quad pK = 25.52 \qquad (15)$$

$$Hg_2Cl_2 \rightarrow 2Cl^- + Hg_2^{++} \quad pK = 17.96 \qquad (16)$$

$$Hg \rightarrow Hg^{++} + 2e \quad E_0 = +\,0.612 \text{ vs. S.C.E.} \qquad (17)$$

$$2Hg \rightarrow Hg_2^{++} + 2e \quad E_0 = +\,0.547 \text{ vs. S.C.E.} \qquad (18)$$

For the purpose of constructing the diagram for use in establishing optimum titration conditions, it is desirable to obtain a line representing the system at a point prior to the end point—half way to the end point, for example—and another line at a point after the titration—as far past the end point, for example, as the other line was prior to the end point. These data were obtained experimentally by mixing the proper chemicals in the correct proportions to represent these two situations and then measuring the potential of the system at various pH values. In this manner lines I, II, III, and V were obtained. The remaining lines were calculated on the basis of the data given in Equations 8 to 18. The exact concentrations employed are listed beneath each species in the reactions I to V, each reaction corresponding to the line in Figure 1 bearing the same designation.

Prior to End Point. The most positive potential that may be obtained is ultimately limited by the formation of mercuric oxide. This region is plotted in Figure 1 from the data given in Equations 15 and 17. In practice a complexing agent—e.g., ammonia—is added to prevent precipitation of mercuric oxide and the potential limit is consequently even more negative. In the titration of metal ions which have stability constants with ethylenediaminetetraacetic acid greater than that of mercuric ethylenediamine tetraacetate, the following displacement reaction occurs prior to titration [assuming no "complex effect" (*9*)]:

$$2NH_4^+ + HgY^{--} + M^{++} \rightarrow MY^{--} + Hg(NH_3)_2^{++} + 2H^+ \text{ (below pH 4)} \tag{19}$$

$$NH_4^+ + NH_3HgY^{--} + M^{++} \rightarrow MY^{--} + Hg(NH_3)_2^{++} + H^+ \text{ (above pH 4)} \tag{20}$$

The equilibrium electrode process is then given by the reaction:

$$\underset{(0.001M)}{Hg(NH_3)_2^{++}} + 2H^+ + 2e \rightarrow Hg + \underset{(0.05M)}{2NH_4^+} \tag{I}$$

In agreement with Equation I, the slope of curve I in Figure 1 corresponds to a two-electron, two-proton reaction.

The metal ions, whose stability constants with ethylenediaminetetraacetic acid are less than that of mercuric ion, do not undergo reactions 19 or 20 over the entire pH range. The weaker the chelating power of the metal, the higher the pH must be for extensive conversion. Thus, for example, at pH 9 all metal ions of pK (with ethylenediaminetetraacetic acid) greater than 11 are "leveled" to the species $Hg(NH_3)_2^{++}$ which in turn would be the species actually titrated. Consequently, these metal ions separately titrated would yield identical potentiometric titration curves. When mixed, no separate breaks would result but only a final break representing the titration of the sum of these ions.

On the other hand, at pH 4, only metal ions of pK (with ethylenediaminetetraacetic acid) greater than 16 are "leveled" to $Hg(NH_3)_2^{++}$, whereas metal ions of pK (with ethylenediaminetetraacetic acid) weaker than 11 are so unstable that they would not titrate at all. Calcium, with an instability constant (pK) of 10.70 (ethylenediaminetetraacetic acid), serves as a typical example. Between pH values 4 and 9, the potential, at the point where half of the calcium has been titrated, is given by the following electrode reaction:

$$\underset{(0.001M)}{H^+ + Ca^{++}} + \underset{(0.01M)}{NH_3HgY^{--}} + 2e \rightarrow \underset{(0.001M)}{CaY^{--}} + Hg + \underset{(0.05M)}{NH_4^+} \tag{II}$$

As seen in Figure 1, the slope of the experimental potential-pH curve II does correspond to this two-electron, one-proton reaction. The intersection of curves I and II occurs at a pH value (approximately 9) where Reaction 20 is essentially complete towards the right. The intersection of curves II and III occurs at a pH value (approximately 4) where Reaction 20 is essentially complete towards the left. At pH 9, calcium will titrate as effectively as any metal ion of greater stability with ethylenediaminetetraacetic acid. At pH 4, calcium will not titrate.

Curve IV represents the equilibrium reaction:

$$\underset{(0.01M)}{HgY^{--}} + \underset{(0.05M)}{NH_4^+} \rightarrow NH_3HgY^{--} + H^+ \tag{IV}$$

At pH below 4, reaction IV exists to the left; above 4, to the right. The dotted line (curve IV) at pH 4 corresponds to the condition where the concentration of HgY^{--} and NH_3HgY^{--} are equal (0.005M) in a 0.05M ammonium ion solution.

After the End Point. Curve III in Figure 1 corresponds to the situation past the end point by a definite amount and at a pH greater than 4.

$$\underset{(0.01M)}{NH_3HgY^{--}} + (n+1)H^+ + 2e \rightarrow \underset{(0.05M)}{NH_4^+} + \underset{(0.001M)}{H_nY^{n-4}} + Hg \tag{III}$$

Below pH 4, the reaction is illustrated by curve V.

$$\underset{(0.01M)}{HgY^{--}} + nH^+ + 2e \rightarrow \underset{(0.001M)}{H_nY^{n-4}} + Hg \tag{V}$$

End-Point Break. The break in the end point (as well as the extent of the titration reaction) is given by the difference between the upper curves—e.g., I, II—and lower curves (III or V). This end-point region is illustrated for the case of calcium ion titration by the shaded portion of Figure 1. For example, at pH 8.5, the potential will be around 0.1 volt *vs.* S.C.E. at a point in the titration corresponding to half-titration ($CaY^{--} = Ca^{++} = 0.001M$). The potential at an equal point past the end point ($HY^{---} = 0.001M$) would be -0.035 volt *vs.* S.C.E. Metal ions which form more stable ethylenediamine tetraacetate complexes than calcium give approximately the same results at this pH. Using Figure 1, one can also conclude that calcium could not be titrated in solutions as acid as pH 4. Reasonable end-point breaks and the extent of reaction increases at higher pH values and a pH of 7 or greater is necessary for effective titrations. This is based on the arbitrary assumption of 0.1 volt difference between upper and lower lines. At pH of 4 to 4.5 metal ions with stability constants greater than approximately 10^{15} [assuming no "complex effect" (*9*)] can be titrated selectively in the presence of calcium. Thus, for example, a two-component mixture—e.g., lead and calcium—could be analyzed in the same solution by titration at pH 4.5 (for lead) and then changing pH to 8.5 for the titration of calcium.

Interference of Chloride. On the edge of Figure 1 are illustrated the interference levels of chloride ion caused by Reactions 16 and 18. For example, in the presence of $10^{-3}M$ chloride ion, the most positive potential obtainable would be approximately 0.2 volt. Chloride ion at this concentration, therefore, interferes with titrations carried out at pH 7 or less. To carry out a titration at pH 3, the chloride ion should be less than $10^{-6}M$ according to Figure 1. Actually and fortunately, this is not necessary in practice. The potential that actually occurs will be a "mixed potential," with Reactions 16 and 18 competing with Reaction 19. Because the smallest concentration of any species involved in Reaction 19 is $10^{-3}M$ (the latent hydrogen ion concentration would be even greater when solution is properly buffered), Reaction 19 will greatly predominate over Reactions 16 and 18 (if chloride concentration were $10^{-6}M$) in determining the net potential. Only when the titration is 99.9% complete will the opposing concentrations (chloride *vs.* metal ion) be of the same magnitude. If sufficient time were allowed at the beginning of the titration for equilibrium, the chloride ion concentration would be reduced from, say approximately 10^{-6} to $10^{-7}M$, at the expense of generating an equivalent amount of mercuric diamine. The error introduced upon subsequent titration of the mercuric diamine would correspond to approximately $5 \times 10^{-7}M$ metal ion at most—a negligible error.

KINETICS

The potential-pH diagram is a useful graphic summary of the pertinent thermodynamics of the system. From such a diagram valuable predictions can be postulated. For example, in the titration of calcium at pH 8, the potential of the mercury pool at the start of the titration will be in the neighborhood of 0.1 volt *vs.* S.C.E. If we forcibly changed this potential to −0.05 volt, current would flow and the situation in the titration vessel would change until a new state of equilibrium was reached, which would correspond to the exact titration of the calcium. From the number of coulombs required, the quantity of calcium could be calculated. The diagram does not, however, tell the rate of flow of current and thus the length of time required for the analysis. The diagram predicts equilibrium, not rates.

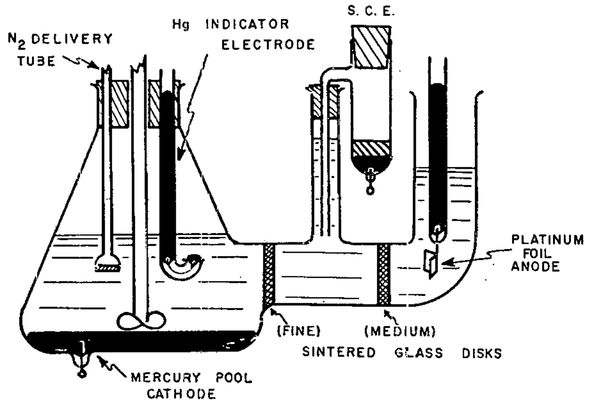

Figure 2. Coulometric titration cell

In the constant-potential coulometric titration the rate of current flow generally would be limited in the most favorable case by the rate of diffusion of the species being titrated. The over-all reaction in the titration of calcium (Equation II) would be controlled by the rate of diffusion of the species on the left of the equation. In practice the solution is well buffered and contains a large excess of NH_3HgY^{--} [ammine(ethylenediaminetetraacetato)mercurate(II)] and, therefore, the diffusion of calcium ions to the surface of the mercury pool might impose the upper limit on the rate of reaction. The limiting factor actually was found to be controlled by a kinetic process in the diffusion layer and, consequently, the over-all rate was much lower. This over-all rate process is studied simply by obtaining a polarogram for this solution. Figure 1 shows that a reduction wave should appear near +0.1 volt *vs.* S.C.E. corresponding to Equation II. The height should be proportional to the concentration of calcium ion if no kinetic effect is operative. A second reduction wave should appear at −0.02 volt *vs.* S.C.E. corresponding to Reaction III, and its height would be proportional to the NH_3HgY^{--} concentration. In practice only the second wave appears.

If the same procedure is carried out at pH 9.3, more favorable results are obtained. The polarogram now shows two steps, the first wave (~ +0.075 volt *vs.* S.C.E.) corresponding to Reaction I in place of Reaction II, and the second wave (~ −0.050 volt *vs.* S.C.E.) corresponding again to Reaction III. Thus by changing pH conditions slightly, a different set of species [$Hg(NH_3)_2^{++}$ and CaY^{--} in place of NH_3HgY^{--} and Ca^{++}] occurs whose electrochemical kinetics is more favorable to rapid reduction. Thus, a combination of potential-pH diagrams and the polarograph as complementary tools is seen to be very useful for establishing best conditions for coulometric analysis at controlled potential.

In the constant current method, the over-all electrode reaction is forced to take place at a given rate. If the desired reaction is too slow, another reaction will occur simultaneously and the entire process may not allow stoichiometric calculations through Faraday's law. In practice the electrode process is never controlled by diffusion of the species being titrated, since this rate would be less than the current flow in the neighborhood of the end point. Therefore, the electrode reaction is arranged such that the product(s) of the electrode reaction will react in solution with the species being titrated. In this way the same over-all reaction occurs, but in roughly two stages. One occurs at the electrode surface and the other in the bulk of the solution. The electrode reaction must be rapid, but the solution reaction can be as slow as can be tolerated in an analysis. The major electrode reaction in the coulometric titration of calcium at constant current is given by Equation III. Because the rate of this electrode reaction is controlled by the diffusion of NH_3HgY^{--} to the electrode surface, the cell was constructed to enhance diffusion by employing a large electrode surface to solution volume ratio

Table I. Coulometric Titration of Metal Ions with EDTA

Cation	Amount Taken, Mg.	Amount Found, Mg.	Error, Mg.	Error, %	Cation	Amount Taken, Mg.	Amount Found, Mg.	Error, Mg.	Error, %
Cu	3.52	3.48	−0.04	−1.2	Zn (*Contd.*)	13.08	13.17	0.09	0.6
		3.54	0.02	0.6			13.08	0.00	0.0
		3.59	0.07	2.1			13.12	0.04	0.3
		Av. 3.54	0.02	0.6			Av. 13.12	0.04	0.3
	7.05	7.10	0.05	0.7	Pb	10.36	10.36	0.00	0.0
		7.00	−0.05	−0.7			10.36	0.00	0.0
		7.06	0.01	0.1			10.31	−0.05	−0.5
		Av. 7.06	0.01	0.1			Av. 10.34	−0.02	−0.2
	14.10	14.01	−0.09	−0.6		20.72	20.80	0.08	0.4
		14.10	0.00	0.0			20.90	0.18	0.9
		14.20	0.10	0.6			20.44	−0.28	−1.4
		Av. 14.10	0.00	0.0			Av. 20.72	0.00	0.0
Zn	0.120	0.121	0.001	0.8		41.44	41.73	0.29	0.7
		0.121	0.001	0.8			41.60	0.16	0.4
		0.121	0.001	0.8			41.48	0.04	0.1
		Av. 0.121	0.001	0.8			Av. 41.60	0.16	0.4
	0.300	0.309	0.009	3.0	Ca	2.01	2.02	0.01	0.5
		0.309	0.009	3.0			2.03	0.02	1.0
		0.301	0.001	0.8			2.00	−0.01	−0.5
		Av. 0.306	0.006	2.3			Av. 2.01	0.00	0.0
	3.27	3.28	0.01	0.3		4.93	4.96	0.03	0.6
		3.30	0.03	0.9			4.97	0.04	0.8
		3.28	0.01	0.3			4.96	0.03	0.6
		Av. 3.29	0.02	0.6			Av. 4.96	0.03	0.6
	6.54	6.58	0.04	0.6		9.86	9.89	0.03	0.3
		6.56	0.02	0.3			9.95	0.09	0.9
		6.49	−0.05	−0.7			9.96	0.10	1.0
		Av. 6.55	0.01	0.1			Av. 9.93	0.07	0.7

and providing efficient stirring action. With these two factors fixed, it was then desirable to find the critical lower concentration necessary to yield 100% current efficiency—that is, to prevent simultaneous generation of hydrogen. This concentration for the current employed was found by following the potential of the mercury pool as measured amounts of the stock mercuric–ethylenediamine tetraacetate solution was added. At the critical concentration the potential shifts suddenly towards a much more positive potential. A more detailed discussion of this procedure is given elsewhere (*1*, *2*, *4*). The concentration necessary was $7 \times 10^{-3}M$. In practice a threefold excess was added to ensure against any transient effects such as lower stirring action.

APPARATUS

A specially constructed titration cell was used (see Figure 2) with a mercury pool cathode and a platinum foil anode.

A Leeds & Northrup pH meter (Model 7664) was employed to measure potential reading from the 700-mv. scale. The end-point system consisted of a saturated calomel reference electrode and a mercury J-tube as an indicator electrode in which a drop of mercury is held in a cup connected through an enclosed platinum wire to a tube of mercury. The constant current supply and associated timer are described in detail elsewhere (*7*). The current magnitude was 43.49 ± 0.04 ma.

REAGENTS AND SOLUTIONS

All chemicals were reagent grade.

The standard copper solution was prepared by dissolving an accurately weighed quantity of electrolytic sheet copper in 5 ml. of concentrated nitric acid and diluting to 250 ml., producing a 0.02*M* standard solution.

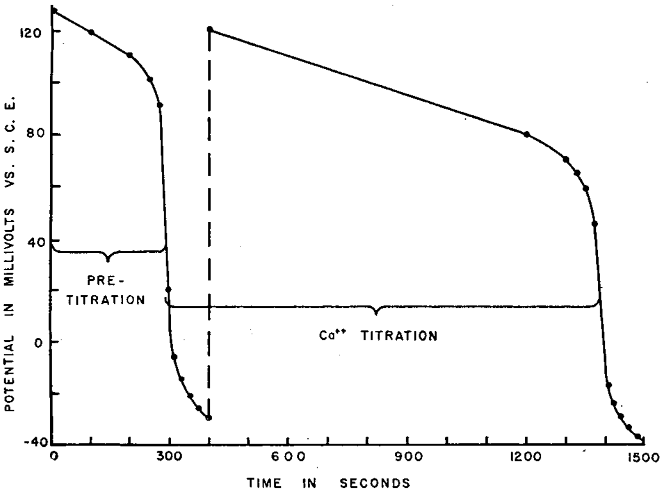

Figure 3. Typical titration curve for calcium

The standard zinc solution was prepared by dissolving 0.3271 gram of 30-mesh granular zinc metal in 5 ml. of concentrated nitric acid and diluting to 250 ml., producing a 0.02001*M* solution.

The standard lead solution was prepared by dissolving 1.656 grams of lead nitrate in 250 ml. of water, producing a 0.02000*M* solution.

The standard ethylenediamine tetraacetate solution was prepared by dissolving approximately 18.6 grams of disodium dihydrogen ethylenediamine tetraacetate (Bersworth Chemical Co.) in 1 liter of water and standardizing this solution against the standard lead solution at a pH of 9, using Eriochrome Schwarz T indicator and a tartrate buffer.

The standard calcium solution was prepared by dissolving approximately 0.9 gram of calcium nitrate monohydrate in 200 ml. of water and standardizing this solution against the standard ethylenediamine tetraacetate solution at a pH of 9, using Eriochrome Schwarz T indicator.

The mercuric ethylenediamine tetraacetate solution was prepared by dissolving approximately 16.8 grams of mercuric nitrate and 18.6 grams of disodium dihydrogen ethylenediamine tetraacetate in 500 ml. of water. This solution (approximately 0.1*M*) proved to have a slight excess of mercuric ion, which was taken into account by the pretitration procedure.

An approximate 0.1*M* ammonium nitrate solution acted as a buffer, as aqueous ammonia was used in bringing each sample to the proper pH.

A saturated potassium sulfate solution was used as an electrolyte in the anode compartment.

PROCEDURE

A 20-ml. portion of mercuric–ethylenediamine tetraacetate stock solution and 55 ml. of ammonium nitrate solution were mixed and placed in the titration cell, which had a mercury pool covering the entire bottom of the flask, and brought to a pH of 8.5 with concentrated ammonia solution. This was done conveniently by adding the base until the potential of the indicator read approximately +0.1 to +0.12 volt *vs.* S.C.E. (see Figure 1). Potassium sulfate electrolyte was placed in the side arms of the cell so that the center arm had a higher liquid level than the outer arm, and both were higher than the solution level in the flask. The mercuric–ethylenediamine tetraacetate solution was then bubbled out with nitrogen for about 10 minutes to remove dissolved oxygen. Failure to remove oxygen was found to give high results. The excess mercuric ion was then pretitrated, using 100-second intervals in the beginning and 10-second intervals when the change in voltage revealed an approaching end point.

A sample of metal ion was than pipetted into the titration cell, and the pH was again adjusted by adding concentrated aqueous ammonia to the solution, while in the titration cell, until a potential of approximately +100 mv. was reached (see Figure 1). The solution was bubbled out again with nitrogen for about 5 minutes, and the sample was then titrated using the same time intervals as in the pretitration. When an end point was reached, the pretitration and the sample titration were plotted and the time interval between the two end points was found as illustrated in Figure 3.

The current was checked every 50 to 100 seconds to ensure its remaining constant throughout the titration.

The results of the titration of four typical cations are summarized in Table I.

ACKNOWLEDGMENT

This research was supported by the United States Air Force through the Office of Scientific Research of the Air Research and Development Command.

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Received for review September 30, 1955. Accepted January 17, 1956.

Chelating Agents in Nonaqueous Titrimetry

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A nonaqueous titrimetric procedure using chelating agents has been developed for determining metal ions. The procedure has been applied to nickel, cobalt, and copper, with accuracy and precision comparable to those of a strong acid–strong base titration. The use of nonaqueous solvents for chelate titrimetry broadens the field in the same manner as it has broadened the scope of titration of other acids and bases. It opens a new phase of chelate titrimetry which may increase its specificity. By proper choice of chelating agent and solvent it is conceivable that titrations specific for many metal ions or groups of ions may be developed. Analysis by this procedure is rapid and does not require expensive equipment.

CONSIDERABLE work has been done in the past few years on titrimetric methods using chelating agents. Schwarzenbach (*2*) has reported the use of chelating agents to titrate many metal ions. Martell and Chaberek (*1*) have also published in this field.

In many cases, however, the chelating agent does not combine with the metal ion strongly enough to produce titration curves suitable for accurate analyses. Also, some chelating agents form intermediate compounds with metal ions which prevent formation of good titration curves. These difficulties have somewhat limited the applications of this method.

The formation constant for metal chelates depends upon the metal, the chelating agent, and the solvent system. Nonaqueous solutions offer some advantages. First, there is a much wider choice of chelating agents, because many more are soluble in nonaqueous than in aqueous solutions. This choice may permit specificity for certain ions or groups of ions. Second, the equilibrium for chelate formation may be changed by varying the solvent system so that some metals which may not have formation constants large enough for titration in aqueous solutions may be titrated in nonaqueous solutions. The method shows possibilities of being made specific for certain metals or groups of metals, provided the proper choice of chelating agent and solvent is made.

Metal chelates are formed by the displacement of one or more acidic protons of the chelating agent by a metal ion. If the moles of hydrogen ion per mole of chelated metal are determined, then the metal ion concentration can be measured by titrating with a strong base.

To demonstrate the applicability of the method to accurate metal analyses, nickel, cobalt, and copper were each titrated using four different chelating agents: dimethylglyoxime, dithizone, 8-quinolinol, and 1-nitroso-2-naphthol. The solvent combination used was 1 volume of methanol to 4 volumes of benzene. The titrant was standard 0.1*N* sodium hydroxide in 1 part of methanol and 4 parts of benzene.

REAGENTS AND APPARATUS

Standard sodium hydroxide, 0.1*N*, was prepared by adding the proper amount of 50% aqueous sodium hydroxide to 1 part of methanol, then adding 4 parts of benzene. The solution was protected from the air with a carbon dioxide absorbent.

Nickel(II) chloride, reagent grade.
Copper(II) chloride, reagent grade.
Cobalt(II) chloride, reagent grade.
Dimethylglyoxime.
8-Quinolinol.
Dithizone.
1-Nitroso-2-naphthol.
Leeds & Northrup pH meter with a glass-calomel electrode system.

PROCEDURE

The aqueous sample containing approximately 2 mmoles of a metal chloride was evaporated on a steam bath. When the sample was dry, 5 ml. of methanol was added and the beaker swirled until the sample had completely dissolved. An excess of the appropriate chelating agent was added followed by 20 ml. of benzene. The solution was then titrated potentiometrically on a Leeds & Northrup pH meter, with the standard 0.1*N* sodium hydroxide in methanol-benzene.

EXPERIMENTAL

Nickel. This procedure was applied to 0.1877*M* nickel chloride solution which had been standardized by a dimethylglyoxime gravimetric analysis. Dimethylglyoxime was also chosen as the chelating agent for the titrimetric nickel analysis. Aliquots of the standard nickel solution were evaporated to dryness and analyzed as described above. A typical titration curve is shown in Figure 1, curve 1. The inflection at the equivalence point is very sharp and occurs at the point in which 2 hydrogen ions per mole of nickel have been titrated.

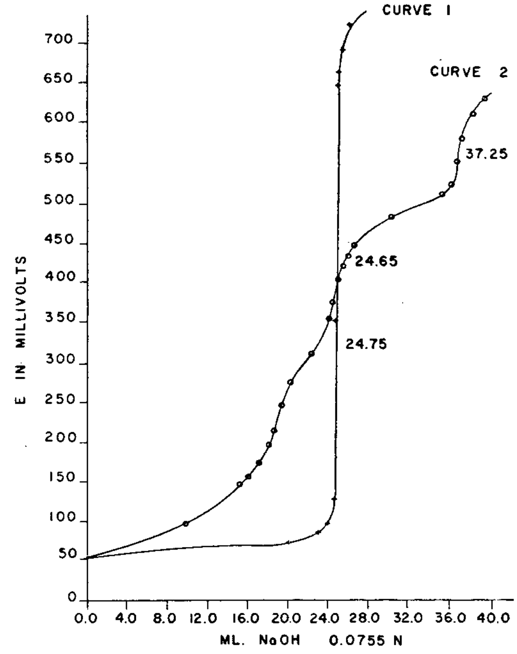

Figure 1. Potentiometric titration of nickel
Curve 1, dimethylglyoxime; curve 2, 8-quinolinol

The analysis was evaluated statistically by doing a series of nine separate determinations. The results are shown in Table I. The precision is comparable to that obtained in aqueous strong acid–strong base titrations.

Equilibrium was obtained rapidly after each addition of base; the potentiometer was steady. The nickel chelate came out of solution but did not interfere with the titration.

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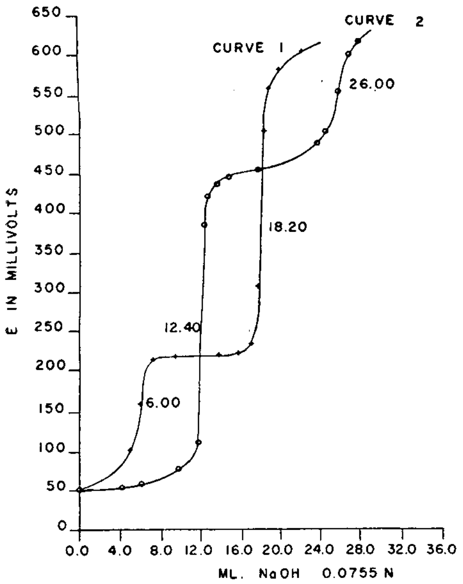

Figure 2. Potentiometric titration of nickel

Curve 1, 1-nitroso-2-naphthol; curve 2, dithizone

A series of titrations was made on the standard nickel chloride solution using 8-quinolinol as the chelating agent. A very different-type curve was obtained as shown in Figure 1, curve 2. The first inflection was not very reproducible; however, the second and third inflections came at points equivalent to 2 and 3 hydrogen ions per mole of nickel and the reproducibility was good.

The mean concentration and the mean deviation found by this method are also shown in Table I.

Figure 2 shows two other titration curves for nickel. Curve 1 was obtained by using 1-nitroso-2-naphthol. The first inflection at 6.00 ml. is approximately equivalent to 1 hydrogen ion per mole of nickel, and the second at 18.20 ml. is equivalent to 3 hydrogen ions per mole of nickel. This inflection is sharp and would be suitable for an analysis.

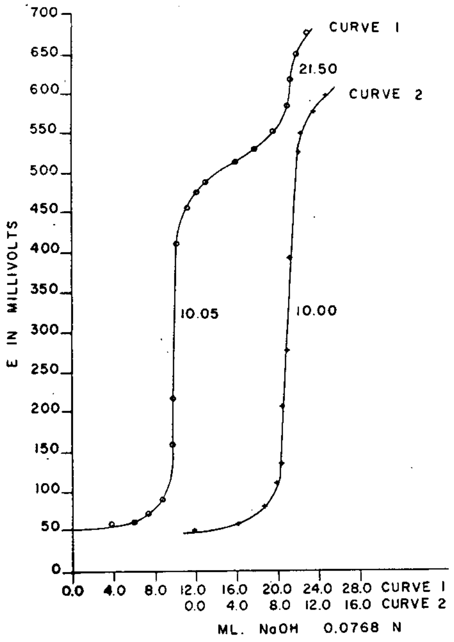

Figure 3. Potentiometric titration of cobalt

Curve 1, dithizone; curve 2, 1-nitroso-2-naphthol

Table I. Titrimetric Analysis of 0.1877M Nickel Chloride Standardized Gravimetrically with Dimethylglyoxime

Chelating Agent	Molarity	Mean Dev., %
Dimethylglyoxime	0.1869	0.2
8-Quinolinol		
2nd inflection	0.1864	0.4
3rd inflection	0.1875	0.2

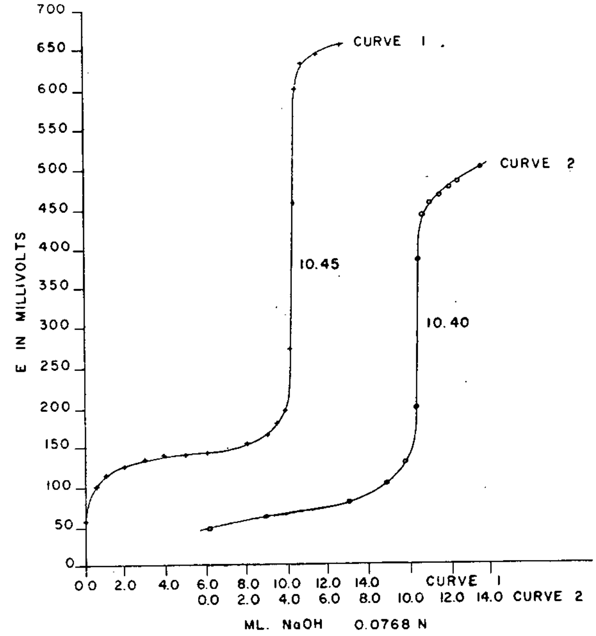

Figure 4. Potentiometric titration of copper

Curve 1, dimethylglyoxime; curve 2, 8-quinolinol

Figure 2, curve 2, shows a titration curve for nickel using dithizone for the chelating agent. Again two inflections were obtained. The first is equivalent to 2 hydrogen ions per mole of nickel and the second equivalent to approximately 4 hydrogen ions per mole of nickel. Here the first inflection was very sharp and would be suitable for an analysis.

Cobalt. A solution of cobalt chloride was prepared and standardized; the concentration was 0.0791M. This solution was analyzed by the titrimetric procedure using each of the four chelating agents. The titration curves obtained with dimethylglyoxime and 8-quinolinol were not suitable for accurate analyses. However, dithizone and 1-nitroso-2-naphthol gave titration curves with sharp inflections at a point equivalent to 2 hydrogen ions per mole of cobalt, as shown in Figure 3.

Copper. A solution of copper chloride was prepared and standardized: the concentration was 0.0800M. This solution was analyzed by the same procedure using each of the four chelating agents. The titration curves obtained with dithizone and 1-nitroso-2-naphthol were poor, but those with dimethylglyoxime and 8-quinolinol were suitable for accurate analyses. The inflections occurred at points equivalent to 2 hydrogen ions per mole of copper (Figure 4).

DISCUSSION

The method described has been applied to the analysis of metal salts in aqueous solutions. The sample aliquot was evaporated to dryness to remove the water plus any excess acid present. However, the method may be applied when a slight excess of acid remains with the metal salt. Analyses have been made on nickel salts containing excess acid, using 8-quinolinol for the chelating agent. The second inflection in the titration curve measures the excess acid plus 2 acid equivalents per mole of nickel. The titrant required for the third inflection then measures only nickel. When dimethylglyoxime is used for the chelating agent, no separation of excess acid and nickel is possible.

The method described was used to determine only one metal ion in a solution. It could equally well be applied to determining the total nickel plus copper plus cobalt in a solution.

It appears probable that two metal ions could be *separately* analyzed—for example, nickel and copper chelated with 8-quinolinol (Figure 1, curve 2, and Figure 4, curve 2) give titration curves which show this possibility.

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Received for review August 26, 1955. Accepted February 4, 1956.

Amperometric Titration of Two- and Three-Component Mixtures of Metal Ions with (Ethylenedinitrilo)tetraacetic Acid

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The combined use of cathodic diffusion current of metal ions and anodic diffusion current of (ethylenedinitrilo)tetraacetic acid under controlled pH conditions made possible the successful amperometric titration of multicomponent mixture. In this way the successive titration of a bismuth-lead-calcium, iron-manganese, or copper-calcium mixture was effected. The results of an experimental survey of the stability of the 15 common metal ions with (ethylenedinitrilo)tetraacetic acid at pH 2, 4, and 9.1 indicate the feasible titration of each under certain conditions. Cobalt(II)-(ethylenedinitrilo)tetraacetate was found to give an anodic wave with a half-wave potential of +0.140 volt *vs.* S.C.E., independent of pH from 4.5 to 10.5. The stability constant of cobalt(III)-(ethylenedinitrilo)tetraacetate was then computed to be $10^{40.7}$ ($\mu = 0.1$). Two new polarographic methods for determining stability constants are described and their limitations discussed.

THREE methods have been reported for the amperometric titration of metal ions with the disodium salt of (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid, EDTA, or Na_2H_2Y). The first method (*8*) utilizes the decrease in height of the reduction wave of the uncomplexed metal ion during the course of the titration.

$$M^{++} + H_2Y^{--} \rightarrow MY^{--} + 2H^{+} \qquad pH = 4 \qquad (1)$$

$$\text{Electrode reaction: } M^{++} + 2e \rightarrow M^{0} \qquad (2)$$

Because of the large stability constants of the metal-EDTA complexes, the half-wave potentials corresponding to the reduction of the free metal ion and its EDTA complex are widely separated, and the selection of the correct potential is not difficult.

The second method (*4*) utilizes the addition of an indicator ion to allow amperometric titrations in cases where the free metal ion does not yield a suitable polarographic wave. For example, in the titration of calcium, zinc ion can be added to act as an indicator because zinc yields a suitable polarographic wave but forms a weaker chelate than calcium in $1M$ alkali. Reaction 3 therefore proceeds in preference to Reaction 4.

$$Ca^{++} + Y^{-4} \rightarrow CaY^{--} \qquad (3)$$

$$2H_2O + ZnO_2^{--} + Y^{-4} \rightarrow ZnY^{--} + 4OH^{-} \qquad (4)$$

$$\text{Electrode reaction: } ZnO_2^{--} + 2H_2O \rightarrow Zn + 4OH^{-} - 2e \qquad (5)$$

The third method (*7*) is based upon the appearance of the anodic wave of free chelating agent (EDTA) after the end point.

$$\text{Electrode reaction: } Hg + HY^{---} \rightarrow HgY^{--} + H^{+} + 2e \qquad pH = 8 \qquad (6)$$

A combination of these techniques permits the successive determination of multicomponent mixtures in certain favorable cases. In this report successful analyses were obtained for the three-component system bismuth-lead-calcium, and for the two-component systems iron-manganese(II) and copper(II)-calcium.

POLAROGRAPHY OF EDTA AND METAL-EDTA COMPLEXES

Before optimum solution conditions and applied potentials could be selected, a study of the polarographic behavior of EDTA and metal-EDTA complexes under a wide variety of solution conditions was required.

Anodic Waves. Goffart, Michel, and Duyckaerts (*2*) and Matyska and Kossler (*6*) have studied the anodic diffusion wave

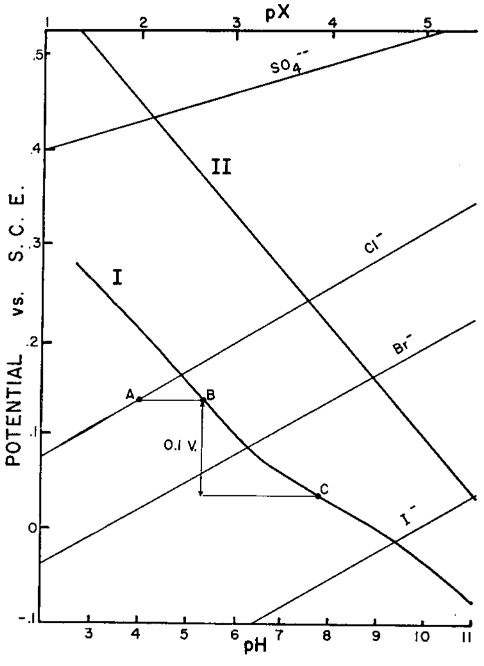

Figure 1. Potential-pH-*pX* diagram for mercury

of EDTA. Because of the discrepancy in the half-wave potentials as a function of pH reported by these investigators, the work was repeated in this laboratory and was found to be in agreement with the data of Goffart, Michel, and Duyckaerts. The half-wave potential of EDTA shifts to more positive values as the pH decreases; therefore, certain ions—e.g., chloride—which form very insoluble precipitates with mercurous ion can be tolerated in higher concentration only for titrations carried out in alkaline solutions. This statement of course applies only where the end point procedure is based upon the anodic wave of EDTA.

A summary of typical interference levels as a function of pH is illustrated in Figure 1. The half-wave potential–pH curve (line I) for the anodic wave of EDTA is plotted from data obtained in this laboratory. The stability constant of mercury(II)–EDTA complex based on this data from pH 4.5 to 9 (μ = 0.1) is $10^{22.12 \pm 0.02}$, in good agreement with the value reported by Goffart, Michel, and Duyckaerts (*2*). The remaining data was taken from Latimer (*5*).

$$\begin{aligned} &Hg_2SO_4 = Hg_2^{++} + SO_4^{--} && K \doteq 1 \times 10^{-6} \\ &Hg_2Cl_2 = Hg_2^{++} + 2Cl^{-} && K = 1.1 \times 10^{-18} \\ &Hg_2Br_2 = Hg_2^{++} + 2Br^{-} && K = 1.3 \times 10^{-23} \\ &Hg_2I_2 = Hg_2^{++} + 2I^{-} && K = 4.5 \times 10^{-29} \\ &HgO(r) + H_2O = Hg^{++} + 2OH^{-} && K = 3 \times 10^{-26} \end{aligned}$$

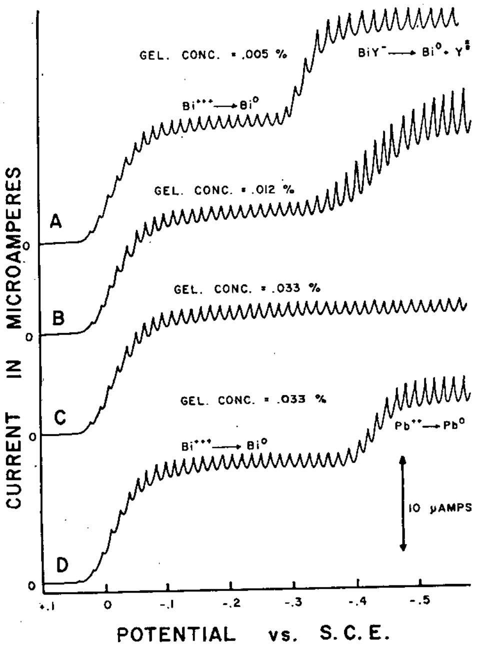

Figure 2. Effect of gelatin on polarographic waves

Polarogram of bismuth ($2 \times 10^{-3}M$)–bismuth–EDTA chelate ($2.3 \times 10^{-3}M$) in presence of

A. 0.005% gelatin
B. 0.012% gelatin
C. 0.033% gelatin
D. 0.033% gelatin, but with lead ion also added

Figure 1 shows that the difference between the half-wave potentials for the anodic wave of EDTA (line I) and the potential corresponding to the formation of mercuric oxide (line II) increases with decreasing pH, being 0.14 volt *vs.* S.C.E. at pH 10 and 0.24 volt *vs.* S.C.E. at pH 3. The presence of buffer materials which form stable mercuric complexes, such as acetate ions or ammonia, decreases this plateau region further at certain pH values. The interference of certain anions is also illustrated: iodide > bromide > chloride > sulfate.

The level of interference may be computed. For example, if chloride ions are present to the extent of $10^{-2}M$ (pX = 2, point *A*), the chloride and EDTA will yield anodic currents at the same potential (+0.135 volt *vs.* S.C.E.) at a pH of 5.3 (point *B*). In order to have at least 0.1 volt potential difference between the two waves, the titration should be carried out at a pH of 7.7 (point *C*) or higher. It is easy to see that titrations carried out at low pH values require careful exclusion of chloride from all reagents. Purification of the EDTA by recrystallization (*1*) was sometimes necessary.

Cathodic Waves. UNUSUAL EFFECT OF GELATIN. A mixture of free metal ion and its corresponding metal chelate may yield two polarographic waves, with the wave for the reduction of the metal chelate occurring at the more negative potential. It has been observed in this laboratory that a certain concentration of surface active agent will decrease and even completely obliterate the wave height for the reduction of certain metal chelates without causing appreciable distortion of the reduction wave of the free metal ions.

Polarographic waves illustrating this effect for a mixture of bismuth ion and bismuth–(ethylenedinitrilo)tetraacetate with varying concentrations of gelatin are shown in Figure 2. The first wave of curve *A* represents the reduction of bismuth ion and the second wave represents the reduction of the bismuth-EDTA chelate; the gelatin concentration is 0.005%. In curve *B*, increasing the gelatin concentration to 0.012% results in a decided distortion of the second wave, and an apparent shift of the half-wave potential to a more negative wave. In curve *C*, increasing the gelatin concentration to 0.033% completely removes the second wave without altering the first wave. In curve *D*, the addition of lead ions to this mixture illustrates that the reduction of lead ions is normal under these conditions.

The mechanism of this effect is currently being studied in this laboratory.

Use of Gelatin for Electrochemical Masking. In an amperometric titration of two metal ions with EDTA—for instance, bismuth ion and lead ion, where the half-wave potential for the reduction of lead ion is more negative than the half-wave potential for the reduction of the bismuth–EDTA chelate—it is important to recognize the useful effect of gelatin concentration. With only the minimal concentration of gelatin (0.005%) present to suppress maxima, the bismuth can be titrated successfully at −0.25 volt with a sharp amperometric end point. The following titration of lead ion at −0.55 volt would not be as successful because the diffusion current of lead ion is superimposed upon the large diffusion current of bismuth–EDTA chelate. By increasing the concentration of gelatin to 0.033% or greater, the diffusion current attributed to bismuth-EDTA complex is eliminated selectively and the concentration of lead ions can be detected with greater sensitivity.

Effect of pH on Formation of Metal–EDTA Chelates. In order to select the metal ions for illustrating multicomponent titrations, the effect of pH on the ease of formation of metal-EDTA complexes was studied. This was achieved experimentally simply by first obtaining an anodic polarogram of EDTA at a specific pH, and then observing the effect of addition of an excess of the particular ion on this polarogram. Figure 3 illustrates some of the results obtained at pH 4. The addition of an excess of lead, manganese(II), bismuth, or nickel ions completely eliminates the anodic wave of EDTA and the resulting polarogram is identical to that of the supporting buffer alone. These metal ions therefore react strongly with EDTA and can be titrated with it at this pH. Calcium and magnesium ions show only partial reaction with EDTA at pH 4 and cannot be titrated satisfactorily. From the polarogram it is evident that EDTA reacts with calcium to a greater extent than with magnesium. This order of reaction (Pb, Mn, Bi, Ni > Ca > Mg) is in agreement with the reported values of their stability constants.

Fifteen metal ions were studied at pH values of 2, 4, and 9.1; the results of this investigation are summarized in Table I. The use of this table is discussed later.

Table I. Effect of Cation on EDTA Anodic Wave

Cation	pH 2, Nitric Acid	pH 4, Acetate	pH 9.1, Ammonia
Aluminum	Weak complex	Forms complex[a]	Forms complex[a]
Bismuth	Forms complex	Forms complex	Forms complex[a]
Cadmium	Very weak complex	Forms complex	Forms complex
Chromium(III)	No complex immediately	No complex immediately	No complex immediately
Cobalt	Forms complex[d]	Forms complex[d]	Forms complex[c]
Copper(II)	Forms complex	Forms complex	Forms complex
Iron(III)	Forms complex	Forms complex	Precipitates
Lead	Weak complex	Forms complex	Forms complex[b]
Manganese(II)	No complex	Forms complex	Forms complex[b]
Nickel	Forms complex	Forms complex	Forms complex
Zinc	Very weak complex	Forms complex	Forms complex
Barium	No complex	No complex	Forms complex
Calcium	No complex	Very weak complex	Forms complex
Magnesium	No complex	No complex	Forms complex
Strontium	No complex	No complex	Forms complex

[a] Forms precipitate which may be prevented prior to titration by addition of triethanolamine.

[b] Forms precipitate which may be prevented prior to titration by addition of tartrate.

[c] Forms precipitate which may be prevented prior to titration by addition of excess ammonia.

[d] Interference caused by anodic wave of Co(II)-EDTA → Co(III)-EDTA + e.

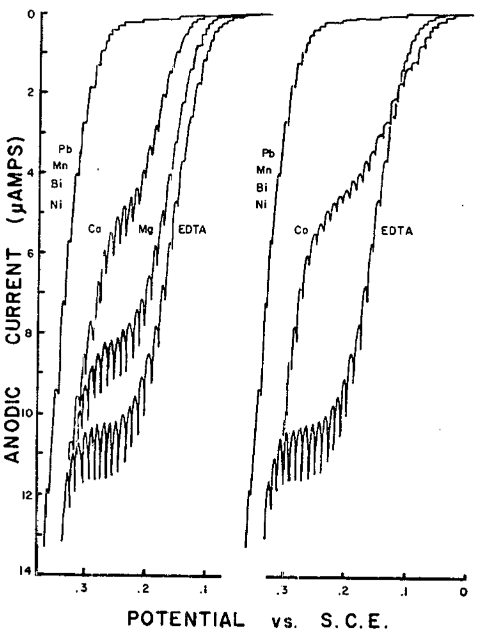

Figure 3. Typical anodic waves obtained in stability study

The general electrode reaction for the anodic wave of EDTA is

$$\mathrm{Hg} + \mathrm{H}_n\mathrm{Y}^{n-4} \rightarrow \mathrm{HgY}^{--} + n\mathrm{H}^+ + 2e^- \qquad (7)$$

when n depends upon pH and varies between 0 and 4. The half-wave potential for the anodic wave of EDTA then becomes

$$E_{1/2} = E^\circ_{\mathrm{Hg}} - 0.0295 \log K_{\mathrm{Hg}}/\Phi \qquad (8)$$

neglecting activity coefficients and assuming that the diffusion coefficients of the mercury-EDTA chelate and the various forms of EDTA (H_4Y, H_3Y^-, H_2Y^{--}, HY^{---}, Y^{-4}) are equal to one another. The term, K_{Hg}, corresponds to the stability constant of the mercury-EDTA complex and $1/\Phi$ to the fraction of the EDTA which is in the Y^{-4} form:

$$\Phi = \left[1 + \frac{[\mathrm{H}^+]}{K_4} + \frac{[\mathrm{H}^+]^2}{K_3K_4} + \frac{[\mathrm{H}^+]^3}{K_2K_3K_4} + \frac{[\mathrm{H}^+]^4}{K_1K_2K_3K_4}\right] \qquad (9)$$

Here K_1, K_2, K_3, K_4 refer to the successive acidity constants of EDTA.

In the presence of an excess of metal ion—for example, lead—the anodic reaction from thermodynamic consideration becomes

$$\mathrm{Hg} + \mathrm{PbY}^{--} \rightarrow \mathrm{HgY}^{--} + \mathrm{Pb}^{++} + 2e \qquad (10)$$

with a half-wave potential corresponding to

$$E_{1/2} = E^\circ_{\mathrm{Hg}} - 0.0295 \log \frac{K_{\mathrm{Hg}}}{K_{\mathrm{Pb}}[\mathrm{Pb}^{++}]} \qquad (11)$$

assuming that the diffusion coefficients of lead and mercury chelates of EDTA are equal and that K_{Pb} corresponds to the stability constant of lead-(ethylenedinitrilo)tetraacetate. The shift in the half-wave potentials as a result of adding an excess of metal ion is, therefore

$$\Delta E_{1/2} = 0.0295 \log \frac{K_{\mathrm{Pb}}[\mathrm{Pb}^{++}]}{\Phi} \qquad (12)$$

At a given pH (Φ is constant at a given pH) and for a given excess of metal ion, the shift in the anodic wave is directly proportional to the stability (pK) of the metal chelate.

Indirect Oxidation Procedure for Determining Stability Constants. In principle this method could be used to determine stability constants of metal ions which do not yield reversible reduction waves, thus extending the application of the polarographic method of stability constant measurements to cases where the customary polarographic method is not applicable. In practice a fiftyfold or greater excess of metal ion would be added, and a pH chosen, thus determining Φ, such that the half-wave potential would occur at potentials more negative than the decomposition potential of the background electrolyte. From the difference in half-wave potentials with and without the metal ion, the stability constant could be calculated using an equation analogous to Equation 12.

Figure 3 points out a limiting feature of this method—namely, that reversible waves will be obtained only if the reaction given in Equation 10 proceeds at a rate much greater than the rate of diffusion of the metal chelate (PbY^{--}) to the electrode. The reaction of CaY^{--} is seen to be slower than that of MgY^{--}, as illustrated by the smaller height at the plateau in the case of CaY^{--}. Michel (*7*) studied this kinetic effect in detail and found that the rates of reaction decreased in general as the stability constants increased. For this reason, no anodic wave corresponding to Equation 10 was found in the cases of lead, manganese, bismuth, or nickel.

In the case of cobalt (Figure 3), an anodic wave was obtained which might be mistaken for weak complex formation. The wave, however, commenced at a slightly more negative potential than the anodic wave of EDTA and its shape was different. A more detailed polarographic study at several pH values showed that the electrode reaction was

$$\mathrm{Co(II)Y}^{--} \rightarrow \mathrm{Co(III)Y}^{-} + 1e \qquad (13)$$

This accounts for the smaller height and elongated shape of the wave, because Equation 13 is a one-electron reaction while Equation 7 is a two-electron reaction. For a one-electron process, $E_{3/4} - E_{1/4} = 0.056$ volt. The average experimental value of $E_{3/4} - E_{1/4}$ for this wave was found to be 0.058 volt. On this basis one may conclude as a first approximation that cobalt(III)-EDTA—cobalt(II)-EDTA is a reversible system. The half-wave potential (+0.140 volt *vs.* S.C.E.) was independent of pH from 4.5 to 10.5 in agreement with Equation 13. The stability constant for cobalt(III)-(ethylenedinitrilo)tetraacetate calculated from this potential is $10^{40.7}$, assuming the stability constant for cobalt(II)-(ethylenedinitrilo)tetraacetate as $10^{16.2}$ (*9*) and the redox potential for cobalt(III/II) as +1.58 volts *vs.* S.C.E. (*5*). The anodic waves for EDTA and cobalt(II)-EDTA have the same half-wave potentials at pH 5. In order to use the anodic wave of EDTA to indicate the end point in a titration of cobalt with EDTA, the pH of the solution must be 7.5 or higher, assuming at least 0.1-volt difference in half-wave potentials is necessary (see Figure 1).

Indirect Reduction Procedure for Determining Stability Constants. The presence of certain metal ions like Ca^{++} may be expected to shift the half-wave reduction potential of a metal chelate such as CuY^{--} to more positive values. In fact, if the free metal ion occurs in smaller concentration than the metal chelate, a prewave is expected whose height would be proportional to the concentration of the free metal ion from equilibrium considerations. Studies on such systems were, therefore, of interest.

An extension of the principle leading to Equation 12 (indirect oxidation procedure) theoretically would allow determination of stability constants for complexes of cations such as Ca^{++}, which is reduced usually only at inaccessibly negative potentials, by an indirect reduction procedure. This procedure in simplified form would involve the determination of the half-wave potentials corresponding to the following systems:

1. Reduction of a simple metal ion: $Cu^{++} + 2e \rightarrow Cu$
2. Reduction of the chelate of the metal ion: $CuY^{--} + nH^+ + 2e \rightarrow Cu + H_nY^{n-4}$, in presence of a large excess of chelate.
3. Reduction of the chelate of the metal ion, $CuY^{--} + H_nY^{n-4}$, in the presence of the other metal ion:

$$CuY^{--} + Ca^{++} + 2e \rightarrow CaY^{--} + Cu$$

The half-wave potential corresponding to System 2 is more negative than that of System 1. The half-wave potential of System 3 lies between that of System 1 and System 2; the greater the stability constant CaY^{--}, the closer System 3 is to the half-wave potential of System 1. The mathematical treatment is simple and readily established for 1 to 1 complexes. The chief disadvantage of this system is that, in practice, reversible waves for System 3 are not obtained because of the relatively slow kinetics involved in the interaction of CuY^{--} with Ca^{++} prior to the faster electrode reaction. Further details are therefore not presented. A related indirect potentiometric procedure has been successfully employed in this laboratory for determining stability constants.

SELECTION OF IONS FOR MULTICOMPONENT TITRATIONS

Consideration of the data in Table I indicates that under proper conditions of pH, any one of the fifteen metal ions could be titrated amperometrically with EDTA. In all cases the end point may be determined by making measurements of the increase in height of the anodic wave of EDTA after the end point has been exceeded, and employing the customary plotting procedure. For some metals the end point may be determined by making measurements of the decrease in height of the reduction wave of the metal ion, as a function of added titrant.

Titrations involving two or more components would be theoretically possible, but experimentally difficult, to perform at the same pH. This difficulty arises from the fact that both species are complexed in the vicinity where the drop of EDTA titrant falls; but then after stirring, the weaker complex reacts only very slowly with the free metal ion that forms the more stable complex. It is possible to avoid this slow attainment of equilibrium by titrating one species of a mixture at a low pH, changing the pH after this end point, and then titrating the second species. An inspection of Table I will suggest several possible combinations. For instance, ferric ion and manganese ion mixtures can be titrated amperometrically with EDTA. A solution of these ions, buffered at pH 2 will allow titration of only the ferric ion. Then by changing the pH to 4, the manganese ion may be determined by measuring the increase in anodic current, caused by excess EDTA.

These same conditions may be applied to three-component mixtures, although now the number of possible combinations is somewhat limited. Again by employing both cathodic measurements and anodic measurements each end point may be determined. Substances such as calcium, magnesium, and barium ions will not form sufficiently stable EDTA chelates in acid solution. Consequently, there are some combinations of metal ions (bismuth, lead, and calcium) that may be titrated in a mixture containing one of these ions.

APPARATUS AND REAGENTS

A Leeds & Northrup Electrochemograph was used for all polarographic studies and for the amperometric titrations. All pH measurements were made with a Leeds & Northrup pH indicator, Type 7664.

Titrants. A standard solution, 0.1070*M*, was prepared from recrystallized EDTA, according to the method of Blaedel and Knight (*1*).

Reactants. Approximately $5 \times 10^{-2}M$ solutions of the nitrates of bismuth, lead, calcium, iron(III), manganese, and copper were prepared. These were standardized amperometrically (*8*), with indicators (*9*), or conductometrically (*3*) using EDTA.

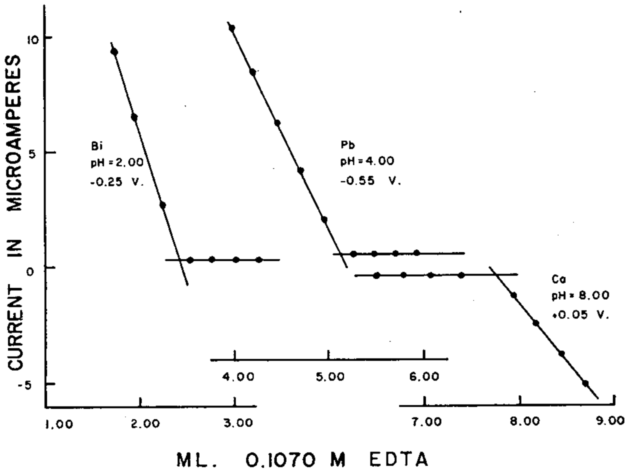

Figure 4. Amperometric titration of a three-component mixture

The solutions of metal ions listed in Table I contained 10 mg. of metal ion per milliliter.

Airco nitrogen, Seaford grade, was used without further purification.

PROCEDURE

To demonstrate the amperometric method for the EDTA titration of multicomponent systems, mixtures of bismuth, lead, and calcium ions were titrated. Bismuth ion forms a stable complex with EDTA at pH 2, while lead and calcium ions do not. Lead complexes strongly at pH 4, while calcium ion remains essentially uncomplexed. Consequently, this mixture may be titrated by changing the pH after the appropriate end points. In order to select the potentials at which current measurements are to be made, it is necessary to obtain polarograms such as those illustrated in Figures 2 and 3. With these conditions established, it is then possible to effect the titration. The change in concentration of bismuth and lead is observed cathodically, and the final increase in concentration of the EDTA, immediately past the calcium end point, is observed anodically. By the plotting procedure, the end points are determined as shown in Figure 4.

TITRATION

Bismuth, Lead, and Calcium. Approximately 0.25 mmole of bismuth, lead, and calcium ion was diluted to 50 ml. and the pH was adjusted to 2.0 with solid chloroacetic acid. A solution of gelatin was added, such that the final gelatin concentration was 0.05%. Care must be taken to avoid contamination of the solution with chloride ions. The decrease in concentration of bismuth ion with added EDTA was determined by making current measurements at −0.25 volt *vs.* S.C.E. After the bismuth ion end point, the pH was adjusted to 4 by adding solid sodium acetate and finally adjusting with 1 to 1 ammonia. Measurements on the decrease in concentration of lead ion were obtained by making current measurements at −0.55 volt *vs.* S.C.E. Similarly, after the lead ion end point, the pH was adjusted to 8 with 1 to 1 ammonia and the calcium ion end point was determined by measurement of the increase in concentration of excess EDTA, as noted by current measurements at +0.05 volt *vs.* S.C.E.

In all cases except where the anodic wave of EDTA is being employed, it is necessary to remove the oxygen from the solution by bubbling with nitrogen for 15 minutes preceding the titration, and for 1 minute after each addition of titrant.

Rather careful control of the pH is necessary for the titration. Above pH 2, the lead ion tends to compete with the bismuth ion for the EDTA. Similarly, calcium ion has a very slight tendency to complex with EDTA at pH 4 (Figure 3). Consequently, the titration of lead ion in the presence of calcium ion should not be carried out in a solution of pH greater than 4.0. Finally, the titration of calcium ion cannot be carried out in a solution of pH greater than 8.0 because of the attack of hydroxyl ion upon the bismuth–EDTA complex. The formation of bismuth hydroxide liberates EDTA, which would then react with the calcium ion present, thus giving a low value for calcium.

Two- and three-component mixtures of these three ions were titrated; the results are presented in Table II. The results for lead are high, possibly because of partial interference of calcium ion. However, neglecting the extreme errors, the results are within the 1% error usually expected in the amperometric method.

Iron(III) and Manganese. The mechanics of the procedure were similar to the one described for the two-component mixture of bismuth and calcium.

Table II. Titration of Bismuth, Lead, and Calcium Mixtures

Present, Mg.			Found, Mg.			Error, Mg.		
Bi	Pb	Ca	Bi	Pb	Ca	Bi	Pb	Ca
54.1	59.1	..	54.6 54.1 54.6	59.4 61.6 60.9	.. .. ..	+0.5 0.0 +0.5	+0.3 +2.5 +1.8	.. .. ..
..	59.1	11.3	.. .. ..	58.7 59.6 59.8	11.6 11.2 11.2	.. .. ..	−0.4 +0.5 +0.7	+0.3 −0.1 −0.1
..	118.2	11.3	..	119.3	11.3	..	+1.1	0.0
54.1	..	11.3	55.0 55.0	.. ..	11.4 11.4	+0.9 +0.9	.. ..	+0.1 +0.1
54.1	..	22.6	55.0	..	22.5	+0.9	..	−0.1
54.1	59.1	11.3	54.1	59.6	11.2	0	+0.5	0
54.1	59.1	22.6	54.8	60.7	22.6	+0.7	+1.6	0
54.1	118.2	11.3	54.3	120.0	11.3	+0.2	+1.8	0
108.2	59.1	11.3	108.2	58.9	11.3	0.0	−0.2	0

The titration of iron(III) was carried out in approximately 50 ml. of 0.8M acetic acid using a dropping mercury electrode at a potential of −0.1 volt *vs.* S.C.E. After this end point, sufficient sodium acetate stock solution (2M) was added to make the solution 0.2M in acetate ions. The manganese was then titrated with EDTA using a potential of +0.2 volt *vs.* S.C.E. to pick up the anodic wave of excess EDTA appearing after all the manganese is titrated. Gelatin was present in a concentration of 0.008%. The results are given in Table III.

Copper and Calcium. The titration of the copper was carried out in approximately 50 ml. of buffer solution consisting of 0.1M ammonia and 0.1M ammonium nitrate. The copper titration curve was obtained by measuring the diffusion current of copper (II)–ammonia complex at −0.25 volt *vs.* S.C.E. After the copper end point the potential was changed to 0.0 volt *vs.* S.C.E. and the calcium was titrated to the appearance of excess EDTA as determined by the appearance of anodic current. The exact end points were determined by plotting the data and locating intersection of the straight lines as usual. Gelatin was present in a concentration of 0.008%. The results of this titration are given in Table IV.

Table III. Titration of Iron and Manganese

Present, Mg.		Found, Mg.		Error, Mg.	
Fe	Mn	Fe	Mn	Fe	Mn
12.8	..	12.4 12.6 12.7 12.6	..	−0.4 −0.2 −0.1 −0.2	..
63.0	..	62.8 63.1 62.9		−0.2 +0.1 −0.1	
..	12.0	..	11.9 12.0	..	−0.1 0
..	62.0	..	62.4 62.5 60.1		+0.4 +0.5 −1.9
12.8	12.0	12.7 12.6	11.3 11.7	−0.1 −0.2	−0.7 −0.3
19.2	6.2	19.0 19.1 19.1	6.8 5.6 6.7	−0.2 −0.1 −0.1	+0.6 −0.6 +0.5
63.0	62.0	63.0 63.2 62.5	62.0 61.9 62.4	−0.0 +0.2 −0.5	+0.0 −0.1 +0.4

Table IV. Titration of Copper and Calcium Mixtures

Present, Mg.		Found, Mg.		Error, Mg.	
Cu	Ca	Cu	Ca	Cu	Ca
13.9	8.80	13.9 13.9 13.9	8.84 8.76 8.86	0 0 0	+0.04 −0.04 +0.06
69.5	44.0	68.7 69.0 69.8	43.7 43.8 43.2	−0.8 −0.5 +0.8	−0.3 −0.2 −0.8

CONCLUSIONS

Utilization of the anodic wave of EDTA provides a convenient method of end point detection for the titration of substances not readily reduced polarographically. No chemical indicator is involved, hence eliminating its associated problems. Furthermore, all of the advantages of the amperometric method apply. For example, measurements need not be made in the vicinity of the end point.

One distinct disadvantage is the necessity for the absence of substances such as chloride ion which would interfere with the EDTA anodic wave for titrations carried out in acid media.

Finally the disadvantage of the amperometric method itself must be considered. The time consumed effecting removal of oxygen, achieving pH changes, and the actual titration is 1 hour for a three-component titration. The removal of oxygen, however, is not necessary if only the anodic wave is used in the titration.

ACKNOWLEDGMENT

This research was supported by the U. S. Air Force through the Office of Scientific Research of the Air Research and Development Command.

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Received for review September 30, 1955. Accepted December 22, 1955.

Film Thickness by X-Ray Emission Spectrography

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When a polychromatic x-ray beam excites the characteristic line of an element in a thin film, the intensity of the line may increase with the thickness of the film, in which case a measurement of line intensity can be used to estimate the thickness. Owing to absorption of x-rays by the film, however, the relationship of intensity to thickness is not generally simple. This absorption filters and attenuates the polychromatic beam, and attenuates the characteristic line. As a consequence, the number of quanta per second contributed to the emergent beam by a layer of atoms decreases with the distance of the layer beneath the surface. Furthermore, quanta originating beyond a critical depth will have no measurable effect on the detector because not enough of them will reach it. For the measurement of film thickness by the method under discussion, this critical depth may be regarded as a critical thickness.

GLOCKER and Schreiber (*4*) appreciated the importance of the critical thickness in x-ray emission spectrography. They pointed out that samples of at least critical thickness, which they estimated to be less than 0.01 cm. for their case, could be considered infinitely thick; for such samples, the measured intensity of a characteristic line must be at the value given by the element in mass. Koh and Caugherty (*5*) proved experimentally that the intensity of a characteristic line emitted by thin metallic films decreases below that for the massive metal as the thickness of the film falls below the critical value, near 0.003 cm. in their case. Brissey, Liebhafsky, and Pfeiffer (*2*) showed by calculation that this value of the critical thickness is reasonable. Koh and Caugherty (*5*) pointed out that x-ray emission spectrography could be used to measure the thickness of films in the range below the critical value. The present investigation is concerned with the basis and the usefulness of this method (Method II) for measuring thickness, and with its relationship to another, Method III, in which thickness is established by the extent to which the film attenuates a characteristic line of the substrate. (Method I differs from Method III in that no analyzing crystal is used.) The designation of the methods is taken from an earlier paper (*11*).

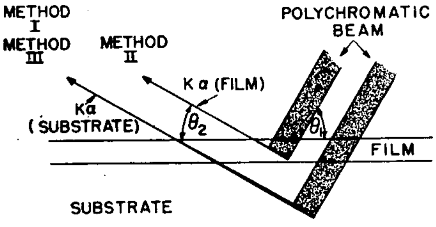

Figure 1. Schematic diagram of three x-ray methods for film thickness

The basis of Method II may be deduced from Figure 1. To do this, the ideal case is considered, in which the x-rays involved are monochromatic, all influences of composition are absent, the simplest optics obtain, and excitation of a characteristic line in the film by a characteristic line of the substrate does not occur. Suppose now that a beam of intensity I_o falls upon a metal film d cm. thick to excite a characteristic line of intensity I_d. The contribution to I_d of a volume element of constant area and of thickness dx, located at depth x, is

$$dI = kI_0e^{-[(\mu_1/\sin\theta_1) + (\mu_2/\sin\theta_2)]\rho x}\,dx \qquad (1)$$

where

k is a proportionality constant that measures the conversion of incident to characteristic radiant energy
μ_1 and μ_2 are mass absorption coefficients for the incident wave length (λ_1) and the characteristic (λ_2)
ρ is the density
θ_1 and θ_2 are the angles made by the incident and emergent beams with the (plane) sample surface (Figure 1)
x is the depth (distance perpendicular to surface).

The integrated equation may be written

$$I_d = k\,I_0\int_0^d e^{ax}\,dx = k\,I_0(e^{ad} - 1)/a \qquad (2)$$

where

$$a = -(\mu_1/\sin\theta_1 + \mu_2/\sin\theta_2)\rho \qquad (3)$$

At infinite thickness ($d = \infty$),

$$I_\infty = -kI_0/a \qquad (4)$$

At the critical thickness d_C, the ratio

$$I_d/I_\infty = 1 - e^{ad} \qquad (5)$$

Table I. Capabilities of Method II

Region	e^{ax}	d or x	dI/dx
Useless	Negligible	Greater than d_C	Approaches zero
Exponential	Significant	Intermediate	Variable
Linear	Near unity	Smaller than x_L	Approaches constancy

The capabilities of Method II can be assessed from Equations 1, 3, and 5 with the results shown in Table I, in which three regions are defined according to the value of the exponential term in Equation 1.

No description of the useless region is necessary in light of the discussion that has been given of the critical thickness d_C.

The linear region is of particular interest. Inasmuch as the relationship of thickness and intensity is made complex by the absorption of x-rays in the film, it seems logical to expect a simplification in the region where the coatings are so thin that absorption is negligible. Intuitively, one is led to surmise that the intensity of the characteristic line in this region will be proportional to the number of atoms in a coherent film, and hence to film thickness. The correctness of this surmise may be demonstrated by examining Equation 1. As x becomes smaller and smaller, the exponential term in this equation increases to the value unity. Once this increase has gone far enough to make the term indistinguishable experimentally from unity, then the term itself may be regarded as constant; whence

$$\Delta I = kI_0\,\Delta x \qquad (6)$$

for all values of x below x_L, the value at which the relationship of intensity to thickness first becomes linear.

The value of x_L depends upon the value of a, and upon what variability in the exponential term may be ignored, the latter consideration usually being governed by the precision attained. If 0.99 is chosen as the value of the exponential term for which this consideration holds, then x_L is 10^{-5} cm. when a is -1000.

Experimental results in accord with Equation 6 have been obtained by Pfeiffer and Zemany (*8*) and by Rhodin (*9*).

The exponential region obviously lies between x_L and d_C.

The existence of the three regions of Table I for each value of a is clear from Figure 3, which contains curves calculated according to Equation 5 for five values of a, one of which was selected to fit experimental data that will be discussed later.

EXPERIMENTAL

When the counting rate was below about 3000 counts per second, the x-ray tube, which had a tungsten target, was operated at 50 kv. and 50 ma. At higher counting rates, the current was reduced to 5 ma., but the rates were corrected to 50 ma. and recorded. A flat, reflecting lithium fluoride crystal was used throughout.

Background Problem. In this investigation, the background count varies with wave length, and it must be estimated for the wave length of the characteristic line being counted. There is also the special problem of change in the background count from that of the substrate metal to that of the metal in the film as the thickness increases. The relationship of this change to atomic number is complex, for it depends not only on the way in which x-rays are scattered by a given atom but also on the way in which they are absorbed.

Over the thickness range for which Method III is useful (*11*), the coatings are usually thick enough so that the background count may be assumed identical with that of the plating metal in mass at the wave length of the characteristic line of the substrate. In the case of Method II, the situation is more complex for the reasons given above, and the need for a reliable background count increases as the coatings become thinner.

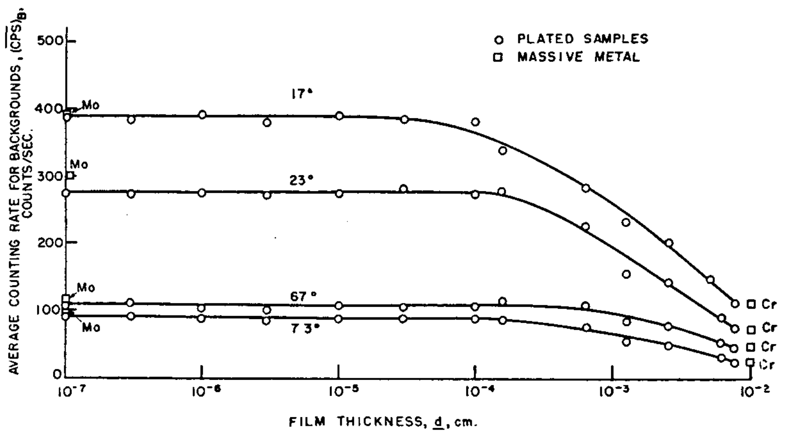

Figure 2. Data for determining background corrections in chromium-on-molybdenum system

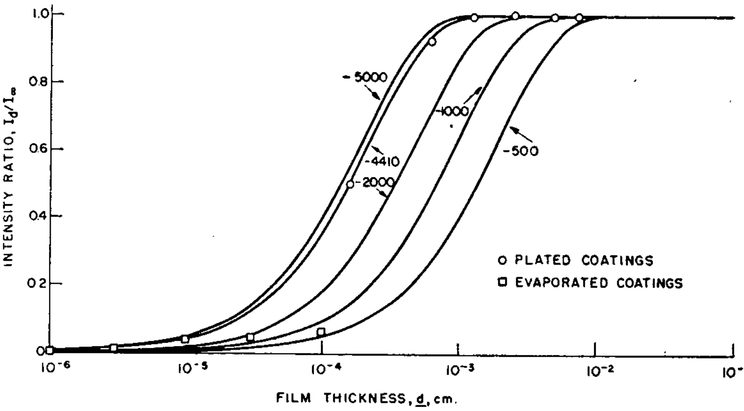

Figure 3. Calculated curves showing relationship between intensity ratio and thickness for various values of exponent a

Figure 2 contains the background data taken on chromium-molybdenum samples for which the thickness of chromium was estimated both by Method II and by Method III. In Method II, the characteristic line of interest is Mo($K\alpha$), at a goniometer setting of 20.255°; background readings were taken at 17° and 23° to bracket Mo($K\alpha$). Similarly, corresponding background values are plotted for Cr($K\alpha$), the characteristic line in Method III, for which the goniometer setting is 69.25°; this line is bracketed between 67° and 73°. The average counting rates for the massive metals are included in Figure 2; trivial discrepancies aside, these rates may be considered for upper and lower limits of the curves for the plated samples.

The background correction at the wave length of the characteristic line was estimated differently in the two cases. For Method III, in which the background correction is always small relative to the counting rate for Mo($K\alpha$), the average rate at the wave length of this line was taken as the mean of the averages for 17° and for 23°. For Method II, the following average counting rates were obtained on uncoated molybdenum: 113.49 (67°); 112.23 [69.25°—i.e., Cr($K\alpha$)]—and 95.87 (73°). Inasmuch as Method II is useful only for very thin coatings, it seemed best to rely on the background relationships obtained for molybdenum in estimating the background correction. Consequently, the 73° values were ignored, and the average background counting rate $(\overline{CPS})_B$ at Cr($K\alpha$) was taken as

$$(\overline{CPS})_B \text{ at } K\alpha = [(\overline{CPS})_B \text{ at } 67°]\ 112.23/113.49 \qquad (7)$$

The foregoing discussion shows that the background correction is important, and that it can be complex enough to require special study.

Chromium on Molybdenum. The importance of protecting high-temperature molybdenum alloys against oxidation is well known. The present investigation was begun in the hope of demonstrating the usefulness of x-ray methods for measuring the thicknesses of metallic coatings on molybdenum alloys.

In order to make available a range of thicknesses that would test both Method II and Method III, thin films of chromium on molybdenum were prepared by evaporation in vacuum and thicker coatings by electroplating. The molybdenum substrate was in the form of disks about 2 cm. in diameter. Those to be coated by evaporation were about 0.05 cm. thick, and those to be plated were about 40 times thicker. Either is, of course, an "infinite" thickness of molybdenum for present purposes.

In neither case is the film thickness known so precisely as in the earlier work with iron foil (*11*). The thickness of the evaporated coatings was calculated from the weight of chromium placed on the filament from which evaporation occurred, geometrical factors being taken into account. In this way, moderately reliable relative values were obtained for the chromium on two pieces of molybdenum at different distances from the source, but the absolute value of the thickness was not well established. Furthermore, the thicker chromium coatings made in this way tended to flake off the molybdenum substrate. With the thickest ones, this trouble was so marked that these were not even measured. Of those measured, some flaking was observed at the two greatest thicknesses, which explains why these points lie below the curve in Figure 3. The thickness of the electrolytic chromium was obtained by careful measurement with a micrometer, but the unavoidable errors here also exceed those attending the use of iron foil.

The experimental results by Method II are plotted in Figure 3 for both kinds of coatings. All average counting rates were corrected for background as described above. The rate for infinite thickness, I_∞, was taken to be the mean of the rates for the four greatest thicknesses measured, which is justifiable inasmuch as these equal or exceed the critical thickness d_C (Equation 5). The ratios I_d/I_∞ were then computed and plotted, and the best-fitting calculated curve belonging to the family in Figure 3 was passed through the plotted points.

Inspection of Figure 3 shows at once that the experimental points fit the calculated curve with two exceptions, the points for the two thickest evaporated coatings, which are too low. The critical thickness is near 0.001 cm., and Method II is obviously useless for estimating thicknesses greater than this.

The curve through the experimental points for chromium-on-molybdenum was calculated by use of $a_{\text{exp.}} = -4410$. Substitution of known values in Equation 3 gives

$$a_{\text{calcd.}} = -(250/0.866 + 110/0.500)\ 6.92 = -3520 \qquad (3a)$$

The mass absorption coefficient 250 is that of chromium for 1.3A., which was taken as the wave length of the incident polychromatic beam (*3*). According to Equation 5, an arithmetic increase in a means an increase in I_d; or, the counting rates measured for the chromium coatings are somewhat higher than one predicts for the simple emission process. This increase could well be due to excitation of Cr($K\alpha$) by the characteristic lines of the molybdenum substrate, notably Mo($K\alpha$).

To test the correctness of this explanation, the Cr($K\alpha$) line was counted (50 kv., 5 ma.) at a later date for the thickest plated sample and for massive chromium. The results: plated sample, 2576 counts per second; massive chromium, 2513 counts per second; background, near 4 counts per second in both cases. Excitation does seem to have occurred as suggested. Metal as a thin plate over the proper substrate can thus yield a more intense characteristic line than the massive metal under the same external excitation. When such strengthening of the characteristic line of the plated metal becomes pronounced, it will have to be considered in formulating the expected relationship between thickness and intensity (Figure 3); the experimental results for chromium on molybdenum are not precise enough to warrant this refinement.

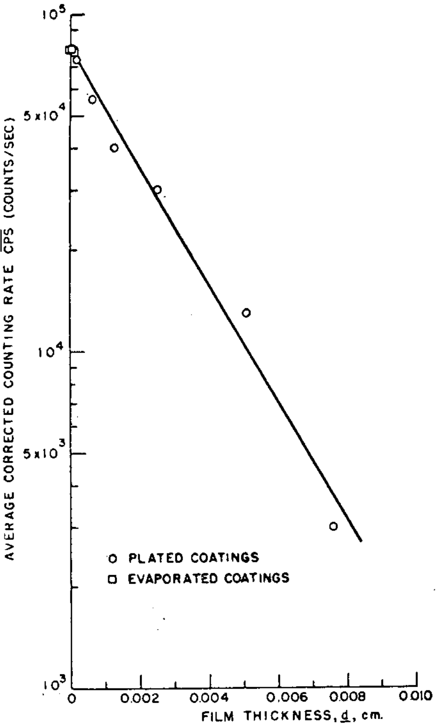

Figure 4. Experimental results for chromium-on-molybdenum by Method III

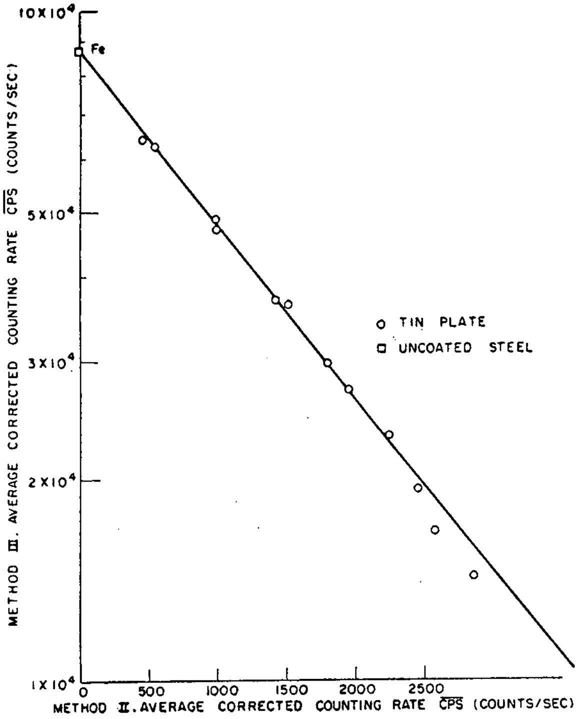

Figure 5. Intercomparison of Methods II and III applied to tin plate

Ordinate scale is logarithmic

No exact test of Equation 6 is possible because the thicknesses of the evaporated coatings are not sufficiently well known. For $a = -4410$, x_L is near 2×10^{-6} cm. under the reasonable conditions postulated when x_L was defined. The four results, two of which could not be plotted in Figure 3, available for this thickness region are in rough agreement with Equation 6. The discrepancies, which are definitely traceable to uncertainties in the method for preparing the films, are great enough to militate against presenting the data. The experiments on tin-plate, discussed later, provide much better support for Equation 6.

Fortunately, Rhodin (*9, 10*) has already presented precise evidence for the applicability of Equation 6 to metallic films at thicknesses near 10^{-6} cm. The work of Pfeiffer and Zemany (*8*), though done on salts deposited on filter paper, also deserves consideration in this connection. Thus, Equation 6 may be regarded as experimentally verified for thicknesses at which reliable standards can be prepared, and it may be used with confidence on thinner films.

It is clear, of course, that the thicknesses discussed here are nominal values that are averages over the area of sample viewed by the detector. They are nominal values because they are calculated by use of the bulk density, which may differ considerably from that of thin films

To establish the relationship of Methods II and III, the samples shown in Figure 3 were run by Method III, which depends upon the attenuation by the chromium coating of the Mo($K\alpha$) line generated in the substrate. Except for the background correction, which was discussed above, the procedure was so similar to the one already developed (*6*) that further description is unnecessary.

The results are plotted in Figure 4, which shows that the samples coated by evaporation are crowded so near the ordinate axis as to be indistinguishable. The electroplated samples, on the other hand, which were indistinguishable in Figure 3, are now grouped satisfactorily around a straight line as the exponential absorption law requires. The divergences from the line are not greater than possible uncertainties attributable to micrometer measurements.

Figure 3 points out that Method II is useful up to thicknesses near 0.001 cm. Figure 4 shows that Method III is useful from that region up to about 0.01 cm., so that the two methods complement each other satisfactorily.

Tin on Steel. Electrolytic tin plate is an excellent material for the present investigation, aside from its great industrial importance. Its production is controlled by Method I (*1*, *7*), and it is highly uniform. The value of a (Equation 3) for tin is near -400, so that x_L is about 2.5×10^{-5} cm., which means that the linear range for tin extends to considerably greater thicknesses than for chromium.

An investigation of tin-plate standards might thus make possible an intercomparison of Methods I, II, and III, and data by Method II might provide additional evidence for the validity of Equation 6. (Method I is essentially Method III with the analyzing crystal omitted.)

Such an investigation was carried out on six standards in the form of panels 6 inches square. These panels were each cut into 24 rectangles, 1 × 1.5 inches. For each standard, one of the 24 rectangles was selected at random and subjected on each face to a measurement of tin thickness by Method II and by Method III. The procedure was generally similar to that for the chromium-on-molybdenum system, with the small difference that the background correction here was calculated for both methods as the mean of appropriate readings—i.e., as in Method III for chromium. Average values by Method I for each face were furnished with the standards.

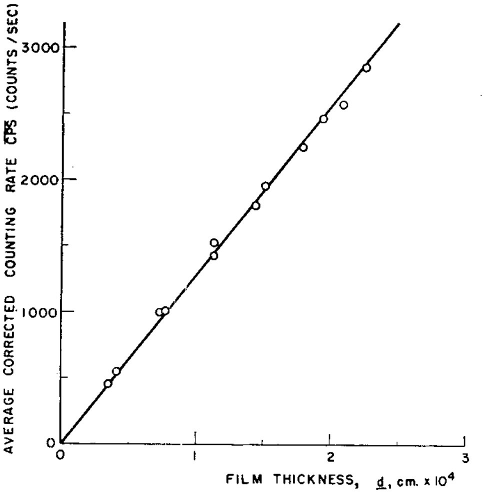

Figure 6. Intercomparison of Methods I and II applied to tin plate

Thickness values based on Method I

The results for the 12 thicknesses studied showed that all 12 were in the thickness region in which both methods were useful; this is in marked contrast to the chromium-on-molybdenum system, for which the overlapping of the useful regions was slight. Owing to the complete overlapping for the tin-plate samples, it is possible to compare these methods by plotting the proper counting rates against each other in such a way that a straight line with an intercept at the counting rate for Fe($K\alpha$) should result if there is complete agreement between the two methods. Figure 5 is evidence that the agreement is good indeed, except at the greatest thicknesses, where the experimental points lie somewhat below the line.

Results by Method II are plotted against known thicknesses (obtained by Method I and based ultimately on chemical determinations) in Figure 6, and results by Method III similarly in Figure 7. Figure 6 shows that Equation 6 is valid up to about 1.5×10^{-4} cm., or considerably beyond 2.5×10^{-5} cm., which was estimated above to be the upper limit of the linear range. The points at the highest thicknesses lie below the line, as would be expected on the basis of Equation 1; this divergence is probably the cause of the deviation from linearity in Figure 5. Figures 5, 6, and 7 do not seem to indicate a clearly greater reliability for any one of the three x-ray methods.

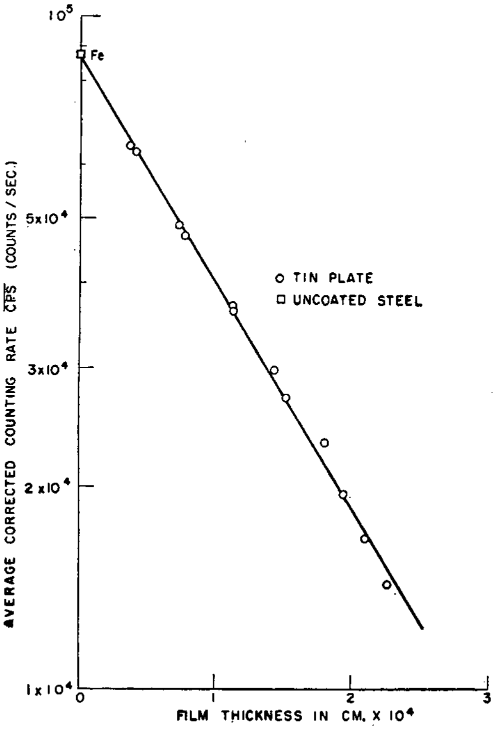

Figure 7. Intercomparison of Methods I and III applied to tin plate

Thickness values based on Method I, ordinate scale logarithmic

Inasmuch as each standard was subdivided into 24 rectangles, only one of which was run, it seemed desirable to test at least one of the standards for uniformity by Method II. This was done by measuring the time for 2^{14} counts at the setting for Sn($K\alpha$) on the same side of each of the 24 rectangles cut from the thinnest tin-plate standard. The standard deviation of these 24 counting periods was 2.1%, and placing these times on a map of the standard showed that their distribution was not random; in other words, the plate was not completely uniform. In agreement with this conclusion, 12 counting periods for one of the rectangles, withdrawn and reinserted between counts, had a standard deviation of only 0.7%. The predicted standard deviation (*6*) for 2^{14} counts is 0.8%, and the close agreement of these two values shows that operating conditions were good.

CONCLUSION

The information given above should make it possible in general to predict the usefulness of x-ray methods in problems involving films. In principle, these methods should be useful occasionally when more than one film is present. The problems in such cases are complex rather than complicated. If both methods are applicable to a duplex film, for example, there will be three characteristic lines to be counted, and absorption effects in three regions to consider. The three counts should, however, contain enough information in many cases to permit the drawing of valid conclusions.

ACKNOWLEDGMENT

The authors wish to thank G. E. Pellissier, Physics and Analytical Chemistry Division, Applied Research Laboratory, United States Steel Corp., for the set of tin-plate standards; and Virginia Thomas and Gloria Kowaleski for help with the investigation.

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Received for review October 3, 1955. Accepted January 17, 1956. Division of Analytical Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

Determination of Iron by Ultraviolet Spectrophotometry

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An ultraviolet spectrophotometric method for the determination of iron utilizes the absorption of the ferric ion in perchloric acid solution. The system shows excellent stability, good sensitivity, and adherence to the usual absorption laws. The method has been applied to a wide variety of industrial alloys and glass samples containing 0.03 to 0.9% iron. Because no special reagents are needed, iron can often be determined in conjunction with the determination of other elements, and, if desired, the entire sample can be recovered and used for subsequent determinations. Owing to the simplicity and rapidity of the method, it is well suited for routine analyses. The interference of a variety of elements is considered.

The ultraviolet absorption spectra of ferric perchlorate and ferric sulfate solutions have been examined previously for analytical applications (*3*). The ferric sulfate system was felt to be better because of less interferences encountered from other metals. Further investigation has shown, however, that the ferric perchlorate system can be used to advantage in the analysis of a large number of industrial materials. The precipitation of insoluble sulfates is avoided, and the perchloric acid medium is often more desirable when other elements are to be determined on the same solution. In addition, the sensitivity is somewhat greater than the ferric sulfate system.

APPARATUS AND REAGENTS

The following were used: Beckman Model DU spectrophotometer with ultraviolet accessories and 1.00-cm. silica cells; primary standard iron metal (Hach Chemical Co., Ames, Iowa); 70 to 72% perchloric acid; methanol; 0.03% hydrogen peroxide (freshly prepared 1 to 1000 dilution of 30% hydrogen peroxide). The above, and other unspecified reagents, were of c.p. quality.

CHARACTERISTICS OF FERRIC PERCHLORATE SYSTEM

The absorption spectrum of ferric perchlorate in excess perchloric acid is shown in Figure 1. Although the absorption peak is at 240 mμ, 260 mμ is preferred as the wave length for analysis because many other metals interfere less at that point.

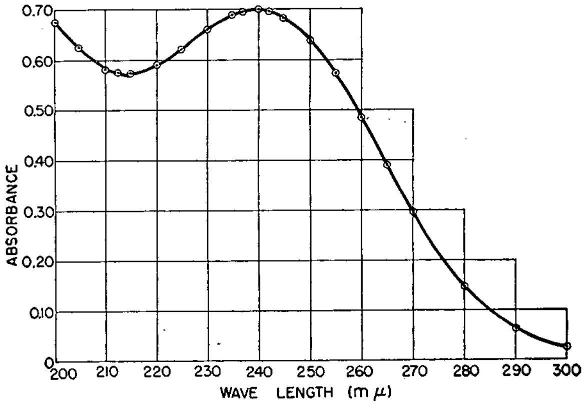

Figure 1. Absorption spectrum of iron(III) in perchloric acid solution

0.93 mg. of iron(III) and 10 ml. of 70% perchloric acid per 100 ml.

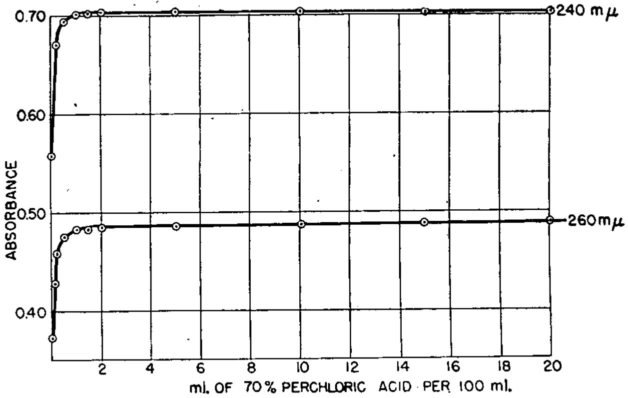

Figure 2. Effect of acidity

Solutions contain 0.94 mg. of iron(III) per 100 ml.

The dependence of the absorption on acidity is shown in Figure 2. The absorption rises until about 1 or 2 ml. of perchloric acid are present per 100 ml., then remains nearly constant up to high concentrations of the acid. At high acidity, the iron is presumably present as free ferric ion.

A concentration of 10 ml. of perchloric acid per 100 ml. was selected for the analyses. Variations of ±5 ml. from this point gave results within the error in photometric reading (±0.2% transmittance), so that a graduate can be used to measure the amount of acid needed.

Adherence of the system to the normal absorption laws is excellent at both 240 and 260 mμ, up to an absorbance of at least 0.8. A typical run over this range at 24° C. gave a molar absorptivity at 240 mμ of $4.16 \times 10^3 \pm 0.03$, and at 260 mμ of $2.88 \times 10^3 \pm 0.02$ liter per mole cm. These data compare favorably with those of Buck, Singhadeja, and Rogers (*4*), who found the peak in 1*M* perchloric acid at 239 mμ and a molar absorptivity of 4.1×10^3 liters per mole cm.

The absorbing species is extremely stable. Several solutions allowed to stand for 24 hours showed no significant change in reading at 240 or 260 mμ. A single solution read at several times over a period of 1 month at 260 mμ showed no significant change.

An increase in temperature decreases the absorbance at both 240 and 260 mμ. The effect is of the order of a few tenths of 1% per degree (22° to 32° C.) at 240 mμ and considerably less at 260 mμ. Temperature is, therefore, unimportant for low percentage work.

ABSORBANCE OF OTHER METALS

Table I shows the absorbance of other metals which, unless otherwise specified, are in the valence state normally attained by fuming with perchloric acid.

Table I. Absorbance of Other Elements[e]

Element	% Element Having Absorbance Equal to 0.001% Fe	
	240 mμ	260 mμ
Ni, Al, Mg. Zn, Cd, Ba, Ca, Na, Mn(II)[a]	>100	>100
Co	80	60
Pb	2.5	>100
Cu	0.072	1.7
Bi	0.018	1.0[d] (about)
Cr	0.0022	0.0014
Cr(III)	1.2[b]	0.60[c]
Ti	0.023	0.035
V	0.0030	0.0031
Mo	0.0033	0.0038

[a] H_2O_2 reduction.
[b] Methanol reduction.
[c] Obtained by both methanol and H_2O_2 reduction.
[d] Beer's law failed at concentrations above 0.4 gram per 100 ml.
[e] Metals used were either iron-free or results were corrected for traces of iron present. Concentrations tested ranged from 2 grams per 100 ml. for the least absorbing to 1 mg. per 100 ml. for the most absorbing.

A number of base metals, including nickel, have an absorption such that, if the material consisted of 100% of the element, an error of less than 0.001% iron would result. Of the two wave lengths, 260 mμ is much more favorable with respect to copper, lead, and bismuth. If large amounts of copper are present, however, a separation is indicated. Vanadium and molybdenum interfere seriously at both wave lengths. The interference of titanium is serious if the ratio of titanium to iron is high, but often this is not the case.

The high absorption of oxidized chromium makes it desirable to reduce this element to the trivalent state. This can be accomplished by boiling the diluted solution with methanol or hydrogen peroxide, neither of which affects the absorption of the iron. The absorption of methanol is so low that it need not be removed; however, hydrogen peroxide does have some absorption and, if a large excess is added, it must be destroyed by boiling. If chromium is present in large amounts, it can be evolved as chromium oxychloride by dropping hydrochloric acid into the fuming perchloric acid solution, taking to fumes again, and repeating several times. The residual chromium is then treated as described above.

Manganese has a very low absorption in the divalent state. When samples containing large amounts of manganese are evaporated to perchloric acid fumes, manganese dioxide may separate. This is readily redissolved by the addition of a little hydrogen peroxide. When samples containing 1 to 5 mg. of manganese per 100 ml. of final dilution were carried through the unmodified procedure, manganese dioxide did not visibly separate. Results for iron were high by not more than 0.01 to 0.06 mg., apparently because of partial oxidation of the manganese to a higher valence. The effect was not reproducible and not always obtained; it was never obtained on solutions containing less than 1 mg. of manganese. Boiling the diluted solution with 1 ml. of methanol or 1 ml. of 0.03% hydrogen peroxide was found to eliminate this effect. No lower amounts of reducing agents were tried because the absorption of these amounts is negligible.

ANALYTICAL PROCEDURES

Absence of Significant Amounts of Manganese and Chromium. The solution should contain about 0.9 mg. (0.3 to 1.5 mg.) of iron and 10 ml. of excess perchloric acid per 100 ml. of final dilution. Unless hydrofluoric acid is present, the solution is taken to perchloric acid fumes in an uncovered 250-ml. Erlenmeyer flask provided with a lip. After cooling, the sides are washed down and the solution is fumed again to expel all volatile acids. The sides are washed down again, and about 40 ml. of water and a few glass beads are added. The contents of the flask are boiled for 1 minute to expel chlorine, and silica is filtered off if necessary. After cooling, the solution is diluted to volume and the absorbance is read at 260 mμ.

Hydrogen Peroxide Reduction. The same procedure is followed, except that after the final addition of water, the solution is heated to boiling and enough hydrogen peroxide is added to dissolve precipitated manganese dioxide in high manganese materials and to reduce chromium present; 1 ml. of 0.03% hydrogen peroxide will eliminate the manganese effect in the range of 1 to 5 mg. Four milliliters of 0.03% hydrogen peroxide have an absorption at 260 mμ equal to 0.01 mg. of iron (about 0.02 mg. of iron at 240 mμ). It was confirmed experimentally that this quantity of peroxide is sufficient to reduce slightly more than 1 mg. of chromium.

The strong color of oxidized chromium often permits limiting the amount of peroxide added. In an otherwise colorless material, after taking to perchloric acid fumes and adding a little water, 0.1 or 0.2 mg. of chromium can be seen. Even in the presence of 1 gram of nickel, comparison of the cooled solution with a nickel sample containing no chromium reveals 0.5 to 1 mg. of chromium.

If a large excess of peroxide is added, it can be destroyed by boiling in the presence of a catalyst. Several metals, including iron, act in this manner.

Tests were conducted on solutions containing 0.3 mg. of iron, 10 ml. of perchloric acid, and 30 ml. of 0.03% peroxide. These were diluted to about 100 ml. in 250-ml. Erlenmeyer flasks and glass beads were added. The solutions were boiled vigorously for 35 minutes, adding boiling water as needed to prevent evaporation to perchloric acid fumes. Readings at 240 and 260 mμ checked with those to which no peroxide was added. In the presence of additional metals, it is likely that this boiling time could be reduced.

Application of this procedure in the absence of any metals indicated that appreciable amounts of peroxide were still undestroyed. Therefore, no peroxide should be added to a blank; or, if desired, a standard can be carried through the entire procedure.

The hydrogen peroxide reduction method is the one most widely employed in this laboratory. Even if chromium and manganese are presumed absent, a little 0.03% peroxide is usually added as a precautionary measure.

Methanol Reduction. The original procedure is followed, except that after the addition of 40 ml. of water, 1 ml. of methanol is added. The solution is heated to boiling, then boiled for 2 minutes. This procedure reduces several milligrams of chromium and eliminates the manganese effect in the range of 1 to 5 mg. Methanol, in the amount of 3.7 ml., has an absorption at 260 mμ equal to 0.01 mg. of iron (4.2 ml. at 240 mμ).

Concentrated perchloric acid or its fumes should not come in contact with alcohol or its vapor. Standard practice in this laboratory is to conduct the methanol reduction in a separate hood reserved for organic materials, and on an electric hot plate. As a further precaution, a dilute solution of methanol might be added, although this was not done in this work.

EFFECT OF REDUCTANTS

The figures given in Table I can be used as correction factors (if the correction introduced is not too large) in cases where interfering elements are presented in approximately known

Table II. Analysis of Cathode Nickel

Heat No.[a]	% Fe Present[c]	% Fe Found		Av. Dev., ± %
		Uncorr.	Corr. for Ti	
H1400	0.036	0.036	0.035	<0.001
		0.036[b]	0.035	<0.001
84	0.060	0.063	0.062	<0.001
202	0.063	0.064	0.063	<0.001
		0.065[b]	0.064	0.001
1025	0.033	0.033	0.033	<0.001
		0.031[b]	0.031	<0.001
8153	0.116	0.113	0.113	<0.001
		0.115[b]	0.115	<0.001
NiO Lot 1	0.39	0.40	0.40	<0.01

[a] One-gram samples of all except NiO, Lot 1, which was 0.25 gram.
[b] Methanol reduction.
[c] Average of results from several different laboratories, chiefly by thiocyanate method.

amounts. If methanol is used as a reducing agent, these factors should remain unchanged. This was found to be true in the case of molybdenum and vanadium.

If excess hydrogen peroxide is added and boiled out, the factors should remain unchanged except for the titanium, molybdenum, and vanadium group. Here, only vanadium is partially reduced, so that about 4 parts of vanadium equal 1 part of iron at both wave lengths.

In order to determine the effect upon titanium, vanadium, and molybdenum of not removing peroxide, from 1.5 to 2.0 mg. per 100 ml. of these elements were treated with 10 ml. of 0.03% hydrogen peroxide in the cold. The readings on vanadium and molybdenum were lowered by about 50% because of formation of peroxy complexes. Although the reading on the titanium solution increased, if the reading due to the hydrogen peroxide alone was deducted, it, too, was decreased by about the same amount. In the presence of 1 ml. of 0.03% peroxide, the titanium correction factor was essentially the same as in its absence.

This suggests a possible method for decreasing the interference of this group by adding, in the cold, the same amount of peroxide to samples and standards. Higher concentrations of peroxide could profitably be tried. Because the interference of the above elements in the samples of interest was small, this subject was not investigated further.

EFFECT OF ANIONS

All volatile anions should be expelled by fuming. Nitrates have a strong absorption in the ultraviolet region. Chlorides and fluorides complex with iron; 10 mg. of fluoride per 100 ml. depressed the absorption of 1 mg. of iron by 32% at 260 mμ, and 17% at 240 mμ. The addition of 0.2 gram of aluminum (as perchlorate) reduced this error to about 1% at 260 mμ, and to less than 1% at 240 mμ, because of the formation of an aluminum fluoride complex (*6*). Boric acid in amounts up to 0.25 gram per 100 ml. had no effect upon the absorption of iron.

Of the nonvolatile anions, 50 mg. of sulfate ion had no effect on 1 mg. of iron at either wave length. Fifty milligrams of phosphate increased the reading on 1 mg. of iron by 3% at 260 mμ and decreased it by about 2% at 240 mμ.

ANALYSIS OF SAMPLES

Most of the analyses have been done without separating interfering elements, unless the interference is appreciable. Each value reported in the following tables represents an average of two or three determinations run simultaneously.

Cathode Nickel. By cathode nickel is meant types 220, 225, and 330 (*5*). These samples contain 99% minimum nickel (plus cobalt) and not more than a few tenths of 1% of copper, iron, manganese, carbon, silicon, magnesium, and aluminum. Titanium occurs in amounts from 0.00% to about 0.04%.

The preferred procedure is the hydrogen peroxide reduction method using 1 ml. of 0.03% hydrogen peroxide. Results on several ASTM round robin samples using this method are given in Table II, along with some results by the alcohol reduction procedure.

A synthetic nickel oxide sample for use as a spectrographic standard was also analyzed by this technique. This sample contains more iron than cathode nickel and about 0.02 to 0.03% chromium.

Metallic samples were dissolved in nitric acid and the oxide sample in aqua regia; final dilutions were 100 ml. Blanks averaged 0.001% iron and were deducted. The correction for titanium is not more than 0.001% iron and is probably within the error of either this or the ASTM thiocyanate method (*1*). The perchlorate method is simpler; no correction is needed for the nickel background, and the same solution can be used for the determination of several other elements.

Silica Base Materials. These samples were treated by the ASTM C169-43 routine method for the determination of R_2O_3, alkalies, and alkaline earths in soda lime glass (*2*), with the exceptions noted. Two-gram samples were dissolved in hydrofluoric and nitric acids in a platinum dish and fumed twice with perchloric acid; 0.5 gram of boric acid was added during the second fuming to help expel fluorides. In preparing the final solution, no hydrochloric acid was added; instead, the required amount of perchloric acid was used. In some cases, the samples were diluted to 100 ml. instead of 200 ml. In the case of sample 102, an aliquot was taken and diluted further.

The samples contained very little manganese, and no chromium was reported. Nevertheless, except in the cases noted, 1 to 2 ml. of 0.03% hydrogen peroxide were added to guard against traces of chromium. This was not boiled out. In some cases, the solutions were very slightly turbid and were filtered before reading.

Blanks run on the platinum dishes, averaged about 0.004% ferric oxide and were deducted. No corrections were made for titanium, because this was negligible in all cases. The values in Table III seem to be in satisfactory agreement with those obtained by the Bureau of Standards.

NBS silicon metal sample 57 was analyzed by the same pro-

Table III. Analysis of Silica Base Materials

NBS No.	Type of Material	Fe_2O_3, %		Av. Dev., ± %
		Present	Found	
128	Soda-lime glass	0.039	0.040[a]	0.001
			0.043	0.002
80	Soda-lime glass	0.065 (0.057–0.07)	0.059	<0.001
89	Lead barium glass 17.5% PbO, 1.4% BaO	0.049	0.050[a]	<0.001
			0.052	<0.001
93	Borosilicate glass 12.76% B_2O_3	0.076	0.078	0.003
102	Silica brick 0.16% TiO_2	0.66	0.67	<0.01

[a] No peroxide used.

Table IV. Analysis of Aluminum Alloys

NBS No.	% of Other Elements of Interest	% Fe Present	% Fe Found		Av. Dev., ± %
			Uncorr.	Corr.	
85A	Cu 2.5 Cr 0.23 Ti 0.016	0.208	0.21	0.21	<0.01
86C	Cu 7.9 Ti 0.035	0.90	0.91	0.90[a]	<0.01
87	Si 6.2 Cr 0.17 Ti 0.16	0.46	0.47	0.46[b]	0.01

[a] Corrected for Cu and Ti.
[b] Corrected for Ti.

Table V. Analysis of Miscellaneous Alloys

NBS No.	% of Other Elements of Interest	Separation Procedure	% Fe		Av. Dev., ± %
			Present	Found	
157	Cu 72 Ni 18 Zn 9.7	Cu plated out	0.053	0.054	<0.001
169	Ni 77 Cr 20 V 0.018	Cr volatilized as CrO_2Cl_2 with HCl	0.54	0.54	<0.01
162	Ni 66 Cu 29 Mn 2.3 Cr 0.24 Ti 0.20	Fe isolated by single precipitation with ammonia	0.34	0.34	<0.01

cedure; a value of 0.66% iron was obtained against a certified value of 0.65%.

Aluminum Alloys. Table IV shows results on some aluminum alloys. Samples 85A and 86C were dissolved in aqua regia. Hydrogen peroxide was the reducing agent; the visual end point was used with a slight excess added. Sample 87 was dissolved in sodium hydroxide in a nickel crucible. The reaction mixture was transferred to an Erlenmeyer flask with dilute hydrochloric acid. Nitric and perchloric acids were added, the solution was taken to fumes, silica filtered off, and chromium reduced with hydrogen peroxide. An identical result was obtained on another sample which was dissolved in sodium hydroxide in a nickel crucible and taken up in dilute perchloric acid. The iron was oxidized dropwise with permanganate, and the excess permanganate reduced with methanol. In this case only a very small amount of silica separated.

The errors introduced by copper and titanium in the samples are, for most purposes, negligible.

Miscellaneous Alloys. Table V shows results on materials in which separations were made as indicated. The residual chromium in No. 169 was reduced by excess hydrogen peroxide, which was boiled out. Hydrogen peroxide reduction was also used on No. 162. The values given are uncorrected; the results would be the same if the corrections were applied for vanadium and titanium. In the case of Nos. 169 and 162, 1-gram samples were carried through the reduction procedure, and suitable aliquots taken.

Sample 169 was also analyzed without separating chromium; this element instead was reduced with methanol. Uncorrected, a value of 0.56% iron was obtained; corrected for chromium and vanadium, this result was 0.53%.

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Reaction of Hydrogen Peroxide with Complexes of (Ethylenedinitrilo)-Tetraacetic Acid and Nitrilotriacetic Acid

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New color reactions of iron and cobalt with (ethylenedinitrilo)tetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) in the presence of hydrogen peroxide at pH above 10 are reported. Iron-EDTA gives a purple color and iron-NTA gives an unstable brown color upon addition of hydrogen peroxide. Under similar conditions, cobalt-EDTA gives a blue color and cobalt-NTA gives a purple color. It is believed that these colored compounds are peroxy complexes. The results of the tests for all common metals are reported, and the possibilities of applying these color reactions to analysis of iron, cobalt, NTA, and EDTA are discussed. A simple and selective procedure has been developed for the spectrophotometric determination of uranium in the presence of other metals by using a mixture of NTA, hydrogen peroxide, and ammonium hydroxide. NTA complexes most interfering metals and prevents them from forming interfering colors or precipitates. At a wave length of 445 mμ, the use of NTA permits the measurement of uranium in the presence of iron, molybdenum, and vanadium.

THE use of chelating compounds for masking interfering metals has been a subject of continuing interest. (Ethylenedinitrilo)tetraacetic acid and nitrilotriacetic acid have relatively weak power to chelate uranyl, molybdate, vanadate, tungstate, and chromate ions which exist in solution as charged groups instead of free metal cations. Determination of molybdenum, tungsten, and uranium based on this principle has been reported (*4*, *6*, *7*).

Results are given here which show the masking effect of nitrilotriacetic acid on the hydrogen peroxide method for uranium and the interesting color reactions of peroxide with the iron and cobalt complexes of (ethylenedinitrilo)tetraacetic acid and nitrilotriacetic acid.

COMPLEXING INTERFERING METALS WITH NITRILOTRIACETIC ACID IN HYDROGEN PEROXIDE METHOD FOR URANIUM

Several methods for the determination of uranium, based on the yellow color of the uranium peroxide compound, have been reported (*1*, *3*, *8*). None is satisfactory without the separation of interfering substances, except that iron may be complexed with tartaric acid. No previous reference has been found on the use of nitrilotriacetic acid or (ethylenedinitrilo)tetraacetic acid for complexing interfering metals in the determination of uranium with peroxide. A procedure for the elimination of interfering

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Table I. Determination of Uranium in Presence of Foreign Metals

(Uranium taken, 3 mg. in 25 ml.)

Foreign Metal Added[a]	Absorbance		
	No NTA or tartrate added; 445 mμ	NTA and Tartrate Added	
		400 mμ	445 mμ
None	...	0.520	0.260
Fe(II)	Ppt.	0.420	0.260
V(V)	0.21	0.680	0.260
Mo(VI)	0.14	0.640	0.260
Ni(II)	Ppt.	0.520	0.260
W(VI)	0.02	0.540	0.260
Ca(II)	Ppt.	0.525	0.260
Al(III)	Ppt.	0.520	0.260
Mn(II)	Ppt.	0.520	0.260
Pb(II)	Ppt.	0.520	0.260
Ti(IV)	0.004	0.590	0.260
Th(IV)	Ppt.	0.524	0.262
Ba(II)	Ppt.	0.520	0.260
Sr(II)	Ppt.	0.520	0.260
Zn(II)	Ppt.	0.520	0.260
Ag(I)	0.00	0.520	0.260
Cd(II)	Ppt.	0.520	0.260
Bi(III)	Ppt.	0.520	0.260

[a] 3 mg. added.

metals is given here which offers a rapid and convenient method for the determination of uranium.

Reagents. Standard uranium solution, 0.5 mg. of uranium per millimeter, was prepared by dissolving 0.445 gram of uranium acetate, $UO_2(C_2H_3O_2)_2.2H_2O$, in water and diluting to 500 ml.

Nitrilotriacetic acid solution, 0.25*M*, was prepared by adding 11.45 grams of nitrilotriacetic acid to water, adding sufficient sodium hydroxide to dissolve the acid, and diluting to 300 ml. with water.

Ammonium hydroxide, 7*M*.

Hydrogen peroxide, 15%, reagent grade.

Procedure. To prepare the calibration curve, 5 ml. of NTA solution and 0.00, 1.00, 2.00, 3.00, 4.00, and 5.00 mg. of uranium, respectively, were added to each of six 25-ml. volumetric flasks. After mixing with 2 ml. of 7*M* ammonium hydroxide, 2 ml. of 15% hydrogen peroxide solution was added and the solution was diluted to 25 ml. with water. The absorbance was measured with the Beckman Model B spectrophotometer at 445 mμ, using the solution without uranium as the blank.

Uranium in the presence of the foreign metals was determined in a similar manner.

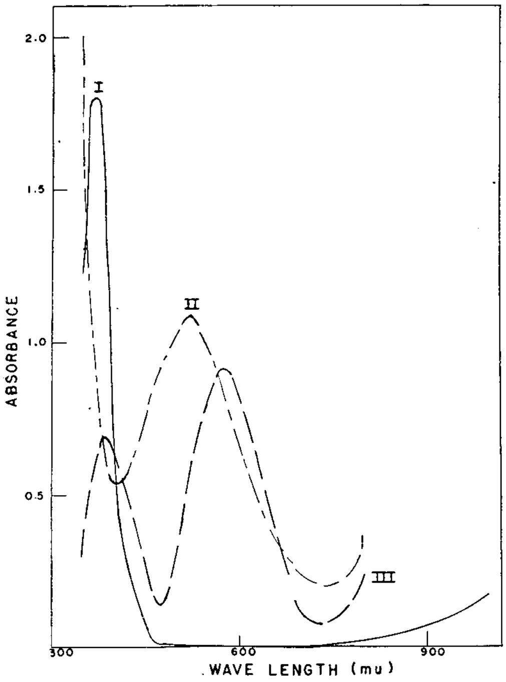

Figure 1. Absorption spectra of metal complexes in presence of (ethylenedinitrilo)tetraacetate and hydrogen peroxide

I. Chromium
II. Iron
III. Cobalt

Results. The calibration curve from the above procedure is shown in Figure 3. Measurements were made at 445 mμ and obeyed Beer's law. The results given in Table I are an indication of the specificity and the reproducibility of the proposed method.

COLOR REACTIONS OF HYDROGEN PEROXIDE WITH IRON AND COBALT

During an investigation in which complexing agents were used to remove interferences in the peroxide method for uranium, it was found that highly colored iron and cobalt complexes were formed upon addition of hydrogen peroxide. Some of their properties and possible applications in analysis were investigated.

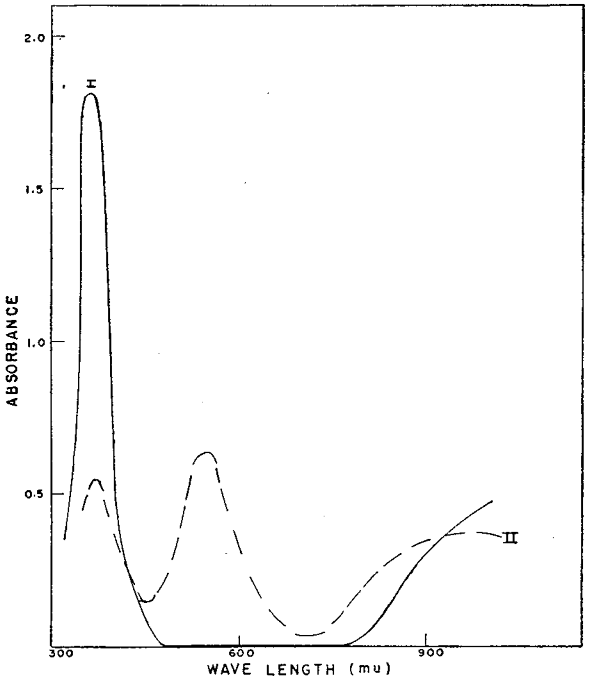

Figure 2. Absorption spectra of metal complexes in presence of nitrilotriacetate and hydrogen peroxide

I. Chromium
II. Cobalt

Iron (ethylenedinitrilo)tetraacetate gave a purple colored complex and iron nitrilotriacetate formed a brown colored complex upon addition of hydrogen peroxide. Cobalt (ethylenedinitrilo)-tetraacetate gave a blue colored complex and cobalt nitrilotriacetate formed a purple colored complex upon addition of hydrogen peroxide. The reactions were made above pH 10. The brown iron complex was very unstable. The other complexes were relatively stable as long as excess hydrogen peroxide was present. It is believed that these colored complexes are peroxy compounds, which may be applied to the determination of iron, cobalt, nitrilotriacetic acid, and (ethylenedinitrilo)tetraacetic acid.

Experimental. ABSORPTION SPECTRA. To 10 ml. of 0.01*M* ferrous ammonium sulfate solution in a 25-ml. volumetric flask were added 5 ml. of 0.1*M* (ethylenedinitrilo)tetraacetic acid and 2 ml. of 7*M* ammonium hydroxide. When 10 ml. of 30% hydrogen peroxide was added, a deep purple color formed immediately. The solution was diluted to 25 ml. with water. The absorbance was measured on the Beckman Model B spectrophotometer using distilled water as a blank. The other peroxy complexes were formed in a similar manner and their absorbances were measured. The absorbance curves are shown in Figure 1.

CALIBRATION CURVES. To each of six 25-ml. volumetric flasks were added 5 ml. of 0.1*M* (ethylenedinitrilo)tetraacetic acid solution and 0.00, 0.50, 1.00, 1.50, 2.00, and 2.50 mg. of iron, respectively. After addition of 2 ml. of 7*M* ammonium hydroxide and 8 ml. of 30% hydrogen peroxide, the solution was diluted to 25 ml. with water. The absorbance was measured on the Beckman Model B spectrophotometer at 520 mμ against the solution which contained no iron.

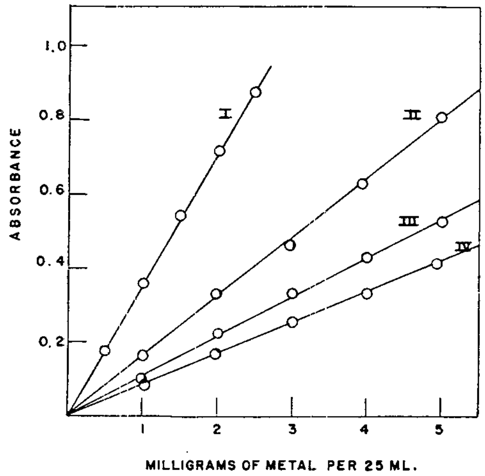

Figure 3. Calibration curves

I. Iron-EDTA at 520 mμ
II. Cobalt-EDTA at 580 mμ
III. Cobalt-NTA at 550 mμ
IV. Uranium at 445 mμ

The calibration curves for the other peroxy complexes were made in a similar manner. They are shown in Figures 2 and 3.

Effect of pH on Color Formation. It was found that the colored peroxy complexes were formed only above pH 10, regardless of whether sodium hydroxide or ammonium hydroxide was used for the pH adjustment.

Stability. The peroxy complexes of iron, cobalt, and chromium were stable in the presence of excess hydrogen peroxide, except iron in nitrilotriacetic acid solution. The iron–nitrilotriacetic acid complex reacted with hydrogen peroxide to form a brown solution, which quickly turned colorless. This made it possible to remove iron interference in the peroxide method for determining uranium.

Table II. Reaction of Metals with Nitrilotriacetic Acid

Metal[b]	NTA–Ammonium Hydroxide	NTA–Tartrate–Ammonium Hydroxide
Au(III)	Reduced	Reduced
Be	White ppt.	....
Ce(III)	Yellow ppt.[a]	Yellow ppt.[a]
Co(II)	Purple[a]	Purple[a]
Cr(III)	White ppt.	Yellow (turbid)[a]
Fe(III)	Brown (unstable)[a]	Brown (unstable)[a]
Mo(VI)	Yellow[a]	Yellow[a]
Sb(III)	White ppt.	....
Sc(III)	White ppt.[a]	....
Sn(II)	White ppt.	....
Ti(IV)	White ppt.	....
UO_2(II)	Yellow[a]	Yellow[a]
V(V)	Lt. yellow[a]	Lt. yellow[a]
W(VI)	Lt. yellow[a]	....
Zr(IV)	White ppt.	....

[a] Formation due to addition of hydrogen peroxide.

[b] No precipitate or color change upon addition of hydrogen peroxide for Ag(I), Al(III), As(III), Ba, Bi(III), Ca, Cd, Cu(II), Eu(III), Ga(III), Ge(III), Hg(III), In(II), Ir(III), Mg, Mn(II), Nb(V), Nd(III), Ni(II), Os(VIII), Pb(II), Pd(II), Pr(III), Pt(III), Rh(III), Ru(III), Se(IV), Te(IV), Tl(I), Y(III), Zn(II).

Possible Applications. The results shown in Figure 3 indicate that the peroxy complexes followed Beer's law; therefore, they could be used for the determination of iron and cobalt. An attempt was made to determine iron in clay samples. Iron could be directly determined in the standard flint clay and plastic clay samples with (ethylenedinitrilo)tetraacetic acid, ammonium hydroxide, tartrate, and hydrogen peroxide without the separation of the interfering substances which are normally present in clay. Furthermore, the characteristic colors of the peroxy complexes serve to identify the presence of nitrilotriacetic acid, (ethylenedinitrilo)tetraacetic acid, and (*N*-hydroxyethylenedinitrilo)triacetic acid (Versen-ol). The two latter compounds gave similar color reactions which indicate their structural similarity.

Reactions with Other Metals. The reactions of other metals with nitrilotriacetic acid and hydrogen peroxide were also tested by adding 1 mg. of the respective cation to 5 ml. of a nitrilotriacetate-tartrate solution, followed by addition of 1 ml. of concentrated ammonium hydroxide and 1 ml. of 30% hydrogen peroxide. (The nitrilotriacetate-tartrate solution was prepared by dissolving 19.1 grams of nitrilotriacetic acid and 23 grams of sodium tartrate dihydrate in 500 ml. of 0.25*N* sodium hydroxide.)

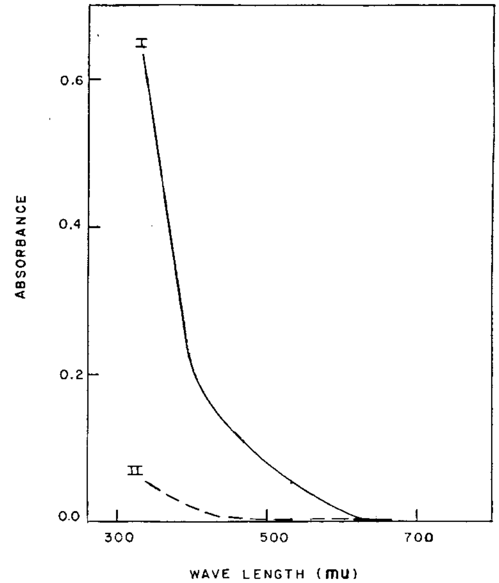

Figure 4. Effect of boiling on absorption of molybdenum complex in presence of nitrilotriacetate and hydrogen peroxide

I. Before boiling
II. After boiling

The results are shown in Table II. The addition of tartrate prevented the precipitation of certain metals such as beryllium(II), titanium(IV), zirconium(IV), antimony(III), and tin(II). Cerium(III) gave a yellow precipitate. Uranyl, molybdate, vanadate, and chromate ions gave yellow colored solutions. Bismuth was oxidized to bismuthate but did not give any precipitate in the absence of cadmium and mercury. The bismuthates of cadmium and mercury are more stable than cadmium and mercury complexes of nitrilotriacetic acid.

DISCUSSION

Previous publications (*1*, *3*, *8*) have shown that the yellow color of the uranium compound developed by peroxide can serve as a quantitative method for uranium and suggest that the intensity of the yellow color be measured at wave lengths from 370 to 400 mμ. They also pointed out that the interfering metals (listed in Table I) must be removed. Tartaric acid has been used for complexing iron (*8*). The use of nitrilotriacetic acid prevented the formation of hydroxides of most metals, including iron. The wave length of 445 mμ was more specific for uranium when the yellow color of the uranium compound was developed in the presence of nitrilotriacetic acid, ammonium hydroxide, and hydrogen peroxide. The wave lengths of 370 to 400 mμ were more sensitive for uranium but less specific, especially when iron, molybdenum, and vanadium were present.

Chromium, vanadium, and iron interfered. The maximum amounts permissible in the presence of 3 mg. of uranium per 25 ml., without causing interference at 445 mμ, are as follows:

	Mg.
Iron	0.5 (as $FeCl_3$) 4.5 (as $FeSO_4$)
Chromium	0.5
Cobalt	3.5

It was found that vanadium interference could not be eliminated by boiling for 10 minutes when ammonium hydroxide was used instead of sodium hydroxide (*8*). However, the molybdenum interference could be eliminated, as indicated in Figure 4, by boiling. There are no absorption maxima in the wave lengths of the visible range for the peroxy complexes of chromium, molybdenum, vanadium, tungsten, and uranium present. But it is possible to select a proper wave length in order to tolerate larger amounts of vanadium, molybdenum, and tungsten (Figure 5). Certainly, the selection of a more specific wave length sacrifices the sensitivity of the method slightly.

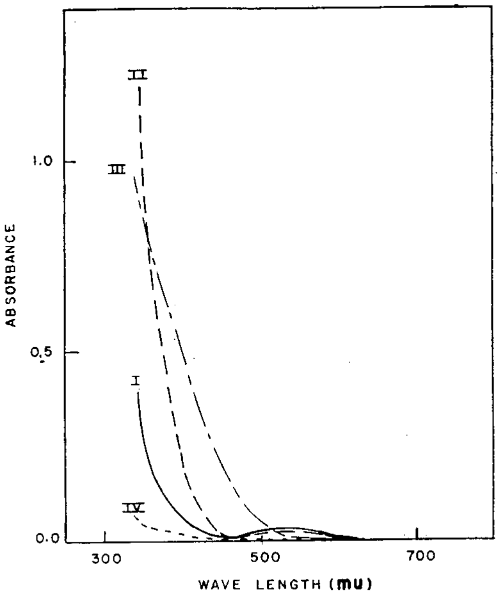

Figure 5. Absorption spectra of metal complexes in presence of nitrilotriacetate and hydrogen peroxide

I. Tungsten
II. Vanadium
III. Uranium
IV. Reagent blank

The addition of peroxide to a mixture of bismuth with cadmium or mercury and nitrilotriacetic acid produced a yellowish precipitate. Presumably, the preciptiate was the bismuthate. But when either cadmium or mercury was absent, no interference occurred. The precipitation of bismuthates in the presence of nitrilotriacetic acid may suggest a new way of isolating and determining cadmium, mercury, or bismuth. The yellow color of the uranium compound developed by peroxide in the presence of nitrilotriacetic acid was very stable and no change in absorbance was observed in a period of 24 hours.

An attempt was made to find if the proposed method could be used as a simple field test for uranium. To a test tube containing approximately 0.5 gram of pulverized carnotite, several drops of 6*N* hydrochloric acid were added followed by 1 ml. of the nitrilotriacetic acid solution and a few drops of 7*M* ammonium hydroxide. The solution remained almost colorless when mixed. On the addition of a few drops of 15% hydrogen peroxide the yellow color of the uranium compound appeared. The color intensity depended upon the amounts of uranium present in the ore sample.

The colored complexes formed by reaction of cobalt-(ethylenedinitrilo)tetraacetic acid complex with hydrogen peroxide have been reported (*5, 9*). No previous reference has been found to the reaction of hydrogen peroxide with nitrilotriacetic acid complexes of iron and cobalt. There are two possible explanations for the formation of the colored complexes. The colored substances are peroxy complexes similar to the peroxyuranates (*10*) or multinucleate amines (*11*); or the metals chelated by (ethylenedinitrilo)tetraacetic acid or nitrilotriacetic acid are oxidized to higher oxidation states, as is the case of the oxidation of (ethylenedinitrilo)tetraacetate complexes of cobalt(II) and manganese(III) to the highly colored complexes of cobalt(III) and manganese(III). Upon addition of hydrogen peroxide to the alkaline solution of iron (ethylenedinitrilo)tetraacetate, iron(III) might be oxidized to iron(VI). The purple color of the iron complex is similar to that of ferrate which has been proved to have iron(VI). The peroxy compounds are usually unstable upon heating. The colored complexes were decolorized by boiling, but the color could be restored by adding additional hydrogen peroxide. Also, the color of the complexes faded slowly on standing if excess hydrogen peroxide was not present, but could be restored by addition of more peroxide. Schwarzenbach (*9*) states that hydrogen peroxide oxidizes the cobalt(II)-(ethylenedinitrilo)tetraacetate complex to cobalt(III) complex with the formula of $(CoYOH)^{--}$. Therefore, it is believed that the (ethylenedinitrilo)tetraacetate (or nitrilotriacetate) complexes of iron and cobalt are the oxidized peroxy compound containing the organic chelating agents. On the other hand, the peroxy compounds of uranium, molybdenum, vanadium, and chromium contain no organic chelating agents. Uranium precipitated in alkaline medium (*2*) in the presence or absence of (ethylenedinitrilo)tetraacetic acid or nitrilotriacetic acid, but the precipitate turned to clear yellow solution upon addition of peroxide. When cyanide was added to the cobalt nitrilotriacetate or (ethylenedinitrilo)tetraacetate solution, the more stable cobalt cyanide complex was formed; therefore, no violet or blue color was formed upon addition of hydrogen peroxide. The colored complexes formed from the reaction of hydrogen peroxide and nitrilotriacetates or (ethylenedinitrilo)tetraacetates of iron and cobalt offer not only new methods for determining iron and cobalt, but also a simple color reaction for differentiating nitrilotriacetic acid from (ethylenedinitrilo)tetraacetic acid or Versen-ol.

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RECEIVED for review June 4, 1955. Accepted January 20, 1956. Division of Analytical Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

Spectrophotometric Determination of Carbonyl Sulfide in Petroleum Refinery Gases

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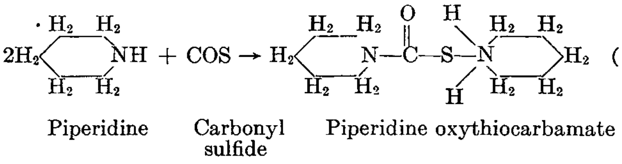
A spectrophotometric method for the determination of carbonyl sulfide in petroleum refinery gases is based on the reaction of carbonyl sulfide with piperidine in aqueous solution to form piperidine oxythiocarbamate. This analysis is performed without any interference from hydrogen, oxygen, nitrogen, carbon monoxide, methane, ethane, acetylene, ethylene, propane, and propylene; and techniques are presented for either removing or correcting interferences that result from the presence of hydrogen sulfide, methyl mercaptan, carbon dioxide, sulfur dioxide, carbon disulfide, thiophene, and conjugated diolefins. An accuracy of 1 p.p.m. of carbonyl sulfide can be obtained for synthetic blends within the range of 1 to 50 p.p.m. The results on refinery samples are reliable to within 10% of the amount present in the range from 1 to 50 p.p.m. of carbonyl sulfide.

IT IS important that the type and quantity of sulfur constituents be known in petroleum refinery gas streams representing feeds from both catalytic and thermal cracking units. Such streams pass from the cracking unit through a Girbotol unit and eventually come in contact with catalyst beds which may be susceptible to sulfur poisoning, depending on the concentration and type of sulfur constituent. A highly sensitive and accurate method has been developed for the quantitative determination of carbonyl sulfide, one of the components that is normally present in varying degrees in cracked refinery gas streams.

Considerable information has been reported concerning the determination of sulfur compounds in gas streams, but only a very few procedures are applicable to the determination of carbonyl sulfide. Brady (*2*) presented a spectrophotometric method for the determination of thiophene, carbonyl sulfide, and carbon disulfide in producer gas. The method is based on the fact that carbonyl sulfide can be scrubbed from a gas stream with a piperidine–ethyl alcohol solution as piperidine oxythiocarbamate, which has a strong absorption band at 230 mμ. Snyder and Clark (*8*) modified the Brady procedure to permit the determination of smaller amounts of carbonyl sulfide. They also describe the use of a manganese dioxide scrubber for eliminating interferences resulting from hydrogen sulfide and sulfur dioxide. An entirely different approach is given by Pursglove and Wainwright (*5*), wherein carbonyl sulfide in synthesis gas is hydrolyzed in dilute base and determined as the sulfide ion by the methylene blue procedure. Other methods appearing in the literature include absorption of carbonyl sulfide and carbon disulfide in a piperidine-chlorobenzene reagent with subsequent colorimetric determination (*6*), oxidation of the carbonyl sulfide with final determination as barium sulfate (*1*), and the application of selective solvents (*3*, *4*).

This method, like Brady's, is based on the quantitative removal of carbonyl sulfide from a gas sample as the piperidine oxythiocarbamate complex, but differs in that an aqueous piperidine solution is used as the extracting medium. The carbamate complex as formed by the reaction following this paragraph is water-soluble in low concentrations, and, when analyzed spectrophotometrically, exhibits strong absorption in the ultraviolet region of the spectrum. A maximum absorption occurs at 230 mμ with a stability period of approximately 18 hours. Beer's law is obeyed over the concentration range studied.



(1)

The method presented gives the advantage of maximum and stable readings immediately after the extraction step, thus eliminating the waiting period of 1 hour or more previously required. Also, the use of water in place of ethyl alcohol as the absorbent increases the sensitivity approximately twofold.

Methods are described whereby the interferences resulting from the presence of hydrogen sulfide, methyl mercaptan, carbon dioxide, sulfur dioxide, carbon disulfide, thiophene, and conjugated diolefins have been eliminated.

REAGENTS

Aqueous 0.043% Piperidine Solution. The extraction solution is prepared by adding 1 ml. of C.P. piperidine to 2 liters of distilled water. This reagent should be protected at all times with a nitrogen blanket to prevent reaction with carbon dioxide or other impurities of the atmosphere.

Shaw's Reagent (*7*). This solution is a mixture of 1 volume of $1N$ sodium carbonate and 7 volumes of aqueous 10% cadmium chloride.

Sodium Hydroxide. Sodium hydroxide (97% minimum) is used to prepare the 30% caustic scrubber solution.

Piperdine Oxythiocarbamate. This material is prepared by bubbling carbonyl sulfide (Matheson Co., Inc., 97%) into 100 ml. of a 5% solution of C.P. piperidine in *n*-hexane. Excess

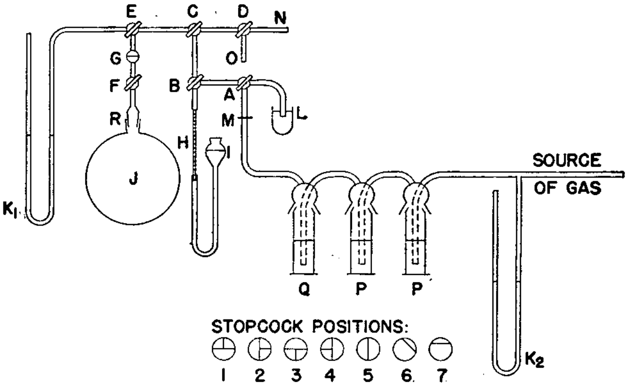


Figure 1. Gas sampling manifold

A, C, D, E.	T-stopcocks
B.	120° stopcock
F.	Straight-bore stopcock
G.	12/5 ball joint
H.	1.00-ml. buret
I.	Mercury reservoir
J.	Calibrated 5-liter sampling flask
*K*₁, *K*₂.	Mercury manometers
L.	Dip tube
M.	Gas sample inlet to manifold
N.	Vacuum line
O.	Vent
P.	Shaw's reagent scrubbers
Q.	30% sodium hydroxide scrubber
R.	45/50 standard taper joint

piperidine is removed from the precipitate by washing with several portions of *n*-hexane. The precipitate is then vacuum dried overnight and stored in a glass-stoppered bottle. Analysis for sulfur confirms the purity of the carbonyl sulfide–piperidine complex.

APPARATUS

A Beckman Model DU spectrophotometer equipped with hydrogen discharge lamp and power supply, and matched 1.00-cm. silica absorption cells was employed.

The gas sampling manifold is shown in Figure 1. Coarse fritted stick-type scrubbing bottles, *Q* and *P*, 125 ml. (Corning Glass Works), are used. All connections to *M* (gas sample inlet to manifold) are made with Tygon tubing; the gas sampling manifold beyond *M* is made entirely of glass. The T-stopcocks *A*, *C*, *D*, and *E*, 120°-stopcock, *B*, and straight-bore stopcock, *F*, are sealed in place in the manifold using 63.5-mm. borosilicate glass tubing. The calibrated sampling flask, *J*, is connected to the manifold by means of a 45/50 standard taper joint, *R*. Any leveling device may be used for raising and lowering mercury in the 1.0-ml. buret, *H*, which is used to introduce known amounts of gases to the sample flask. The mercury manometers, K_1 and K_2, and dip tube, *L*, serve to indicate and aid in controlling the gas pressure and flow in various parts of the system. All joints and stopcocks are lubricated with Apiezon Type N grease.

The special pipet, Figure 2, holds 100 ml. when filled to the mark. Extraction liquid is transferred from the special pipet to the calibrated sampling flask by removing the flask from the gas sampling manifold at *G* (Figure 1) and making the pipet–sampling flask connection with the 12/5 ball joint.

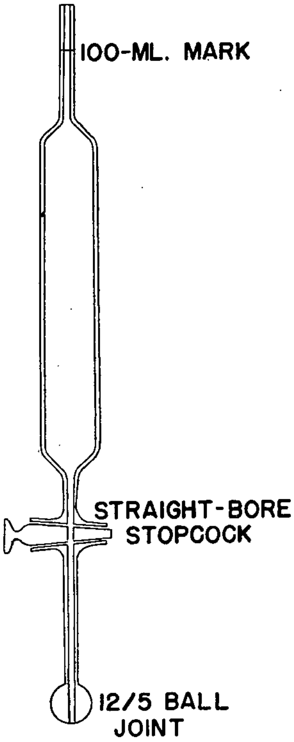


Figure 2. Special 100-ml. pipet

Figure 3 shows the mechanical shaker that was designed to ensure contact between the extraction solution and the gas sample. A $^1/_{25}$-hp. motor supplies the power. The calibrated sampling flask is secured by means of a properly spaced clamp and ring holder. The gas sample and the extraction solution are thoroughly mixed by a vertical pumping motion.

EXPERIMENTAL

The calibration data are obtained by dissolving an accurately weighed amount of piperidine oxythiocarbamate in aqueous 0.043% piperidine solution, diluting aliquot portions of the prepared solution and determining their absorption at 230 mμ with a Beckman Model DU quartz spectrophotometer. Aqueous piperidine is used as a reference. The complex decomposes on standing, and fresh reagent must be prepared as needed.

For an analysis the petroleum refinery gas is passed through the gas sampling apparatus, Figure 1, at a rate of 25 liters per hour. The calibrated flask and the various lines down to *B* are evacuated, as indicated by K_1, by placing *D* in position 1, *C* and *E* in position 3, *B* in position 7, and *F* in position 5. Then, with *A* in position 3, *B* in position 6, *C* in position 2, and *D* in position 4 the petroleum refinery gas is slowly passed around the *A-B-C-D* part of the manifold with a slight flow being maintained through the dip tube, *L*. After sufficient flushing, *C* is turned to position 4, with *E* in position 3 and *F* in position 5. The evacuated flask, *J*, is slowly filled with gas as indicated by K_1 by regulating *F* while preserving a constant flow through dip tube, *L*.

After the calibration flask is filled with refinery gas, it is disconnected from the manifold at *G*. The special pipet, Figure 2, is rinsed with several portions of aqueous 0.043% piperidine extraction solution and then filled to the mark. A small amount of pressure is applied to transfer the extraction solution from the pipet to the calibrated flask, which is then inserted in the mechanical shaker where its contents are mixed vigorously for a period of 80 minutes. Then a portion of the extraction solution is delivered into a 1.00-cm. silica absorption cell and its absorption measured at 230 mμ, using fresh aqueous 0.043% piperidine extraction solution as a comparison blank. Absorption readings are constant immediately following the shaking step. Residual extraction solution is expelled from the flask, which is then rinsed with two successive 100-ml. portions of fresh solution. A final 100-ml. portion of fresh extraction solution is pressured into the flask and the contents are mixed vigorously by shaking for a second 80-minute period. This final solution is analyzed for consideration as a "blank." The difference between the absorbance values of the first and second extraction solutions results in a value which is quantitative for the determination of carbonyl sulfide in petroleum refinery gas.

The pH of the aqueous piperidine extraction solution is approximately 11.2 and it is important that this value be maintained within ±0.2 unit. All glassware, and especially the calibrated sampling flask, should be washed free of acidic impurities. In rare instances it may be necessary to add a second sodium hydroxide scrubber to ensure the removal of acidic impurities in the gas sample. However, this should be done with caution because it increases the possibility of carbonyl sulfide hydrolysis.

Approximately 10% of the carbonyl sulfide hydrolyzes when a gas flow rate of 25 liters per hour is maintained, using 80 ml. of 30% sodium hydroxide reagent in scrubber *Q*, Figure 1, which results in 3.5 inches of reagent above the frit end. It may be necessary to add about 10 drops of Dow Corning Antifoam A emulsion to the caustic scrubber to reduce foaming. The hydrolysis may be decreased even more by increasing the gas flow.

Calculation. The calculation of concentration is made routine by applying

$$\text{Carbonyl sulfide, p.p.m.} = \frac{25{,}600\ (G_1 - G_2)}{V}$$

where

G_1 = grains of carbonyl sulfide per 100 ml. of aqueous piperidine, obtained from the application of Beer's law (or from a calibration curve) corresponding to the absorbance value of the extraction solution at 230 mμ after the first 80-minute period of contact.

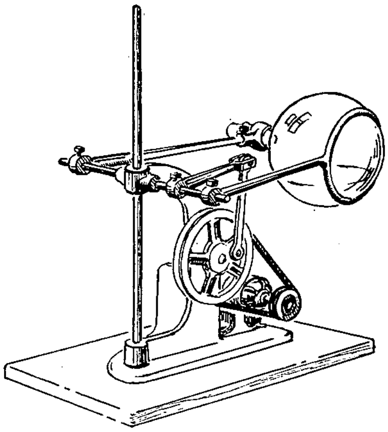


Figure 3. Mechanical shaker

G_2 = grains of carbonyl sulfide per 100 ml. of aqueous piperidine, obtained from the application of Beer's law (or from a calibration curve) corresponding to the absorbance value of the extraction solution at 230 mμ after the second 80-minute period of contact.

V = volume of hydrocarbon refinery gas in liters at 60° F. and 1 atm.

DISCUSSION

The purpose of this investigation was to develop an analytical method, applicable to control work, to be used for determining small concentrations of carbonyl sulfide in petroleum refinery gas samples. At first, the accuracy of the method was proved

Table I. Determination of Carbonyl Sulfide in Inert Gases

Diluent Gas	Carbonyl Sulfide, P.P.M.		
	Added	Found	Diff.
Propylene	7.1	6.7	−0.4
	13.6	12.8	−0.8
	28.8	28.1	−0.7
	31.6	32.0	+0.4
	34.1	34.7	+0.6
	11.8	12.2	+0.4
	51.9	50.5	−1.4
Hydrogen	13.5	12.9	−0.6
	15.1	15.5	+0.4

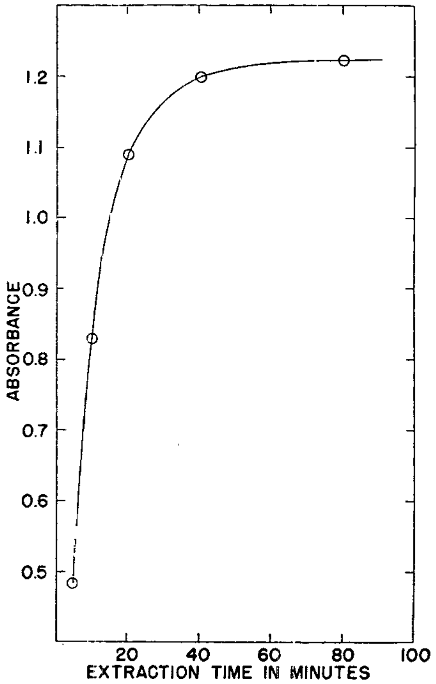


Figure 4. Extraction curve of carbonyl sulfide

Cell length, 1.00 cm.; extraction solution, 100 ml. of aqueous 0.043% piperidine solution. Carrier gas, propane; wave length, 230 mμ; carbonyl sulfide, 40.3 p.p.m. by volume

by determining known concentrations of carbonyl sulfide in inert diluent gases. Table I shows results obtained by adding accurately measured volumes of carbonyl sulfide to the calibrated sampling flask and diluting with propylene or hydrogen.

In order to obtain the accuracy shown in Table I, it is necessary that a contact time of approximately 80 minutes be employed for complete extraction of carbonyl sulfide from the carrier gas (Figure 4). However, it should be emphasized that over 95% of the carbonyl sulfide is extracted in 40 minutes, which may be sufficiently accurate for certain control purposes. The use of the aqueous piperidine results in maximum absorbance readings immediately after the extraction step (Table II).

The sensitivity of the method is almost doubled as a result of substituting aqueous piperidine for piperidine–ethyl alcohol as the extraction solution. Table III demonstrates this fact.

Correction of Interferences. To justify the application of this method for the determination of carbonyl sulfide in petroleum refinery gases, it is first necessary to have a complete understanding of the effect of various constituents that might be present. No interference is exhibited by hydrogen, oxygen, nitrogen, carbon monoxide, methane, ethane, acetylene, ethylene, propane, and propylene. Interference is exhibited at 230 mμ by thiophene, conjugated diolefins, and the piperidine derivatives of hydrogen sulfide, methyl mercaptan, carbon dioxide, sulfur dioxide, and carbon disulfide (Figure 5). Table IV presents information concerning the gas mixtures that were used to obtain the curves shown in Figure 5.

Table II. Stability of Aqueous Piperidine Oxythiocarbamate Solutions

Solution	Absorbance			
	2 minutes	1 hour	5 hours	18 hours
1	0.550	0.552	0.549	0.545
2	0.512	0.512	0.514	0.512
3	0.269	0.267	0.265	0.267

Table III. Aqueous Piperidine *vs.* Piperidine–Ethy Alcohol

Carbonyl Sulfide, P.P.M	Absorbance	
	Aqueous piperidine	Piperidine–ethyl alcohol
4.8	0.149	0.078
9.6	0.290	0.152
14.4	0.435	0.230
24.0	0.723	0.380
38.4	1.154	0.605
48.0	1.442	0.755

The ultraviolet spectrum of a propylene synthetic gas mix containing 19.6 p.p.m. of carbonyl sulfide is represented by curve 1. No attempt was made to extract the interfering gas completely from the propylene carrier gas with the aqueous piperidine solution. The interfering components may be divided into two groups: compounds that are eliminated by scrubbing and compounds that are corrected by application of a "blank" run.

The interferences caused by hydrogen sulfide and methyl mercaptan are eliminated by scrubbing the refinery gas with Shaw's reagent and aqueous 30% sodium hydroxide prior to analysis. The scrubbers containing Shaw's reagent must be supplemented by a caustic scrubber to remove carbon dioxide released by the hydrogen sulfide. Sulfur dioxide and other acidic components are also removed quantitatively by the scrubber containing 30% sodium hydroxide. Large quantities of carbonyl sulfide may be lost by hydrolysis in 30% sodium hydroxide solution if care is not taken. Table V illustrates that the loss of carbonyl sulfide by hydrolysis in the scrubbers is within an acceptable range as long as the flow rate, type of scrubber, and height of scrubbing liquid are properly standardized.

Table IV. Concentration of Interfering Components

Interfering Component	Concn. in Propylene Carrier Gas
Hydrogen sulfide	33.1 p.p.m.
Methyl mercaptan	62.3 p.p.m.
Carbon dioxide	100%
Sulfur dioxide	50.8 p.p.m
Carbon disulfide	52 p p m
Thiophene	206 p.p.m.
1,3-Butadiene	0.05%

Table V. Caustic Scrubbing of Carbonyl Sulfide in Propane-Propylene Synthetic Mix

Carbonyl Sulfide, P.P.M.	
Before scrubbin	After scrubbing
6.0	5.5
8.1	7.3
10.0	9.5
12.6	11.3
15.1	13.6

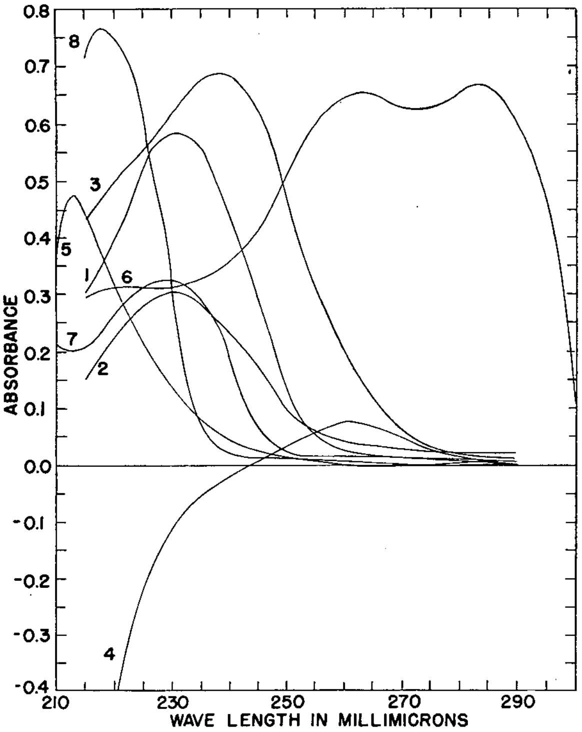


Figure 5. Ultraviolet spectrogram of piperidine oxythiocarbamate and typical interferences

1. Piperidine oxythiocarbamate expressed as carbonyl sulfide

Interferences:

2. Hydrogen sulfide
3. Methyl mercaptan
4. Carbon dioxide
5. Sulfur dioxide
6. Carbon disulfide
7. Thiophene
8. 1,3-Butadiene

Because carbon disulfide, thiophene, and 1,3-butadiene are sparingly soluble in aqueous piperidine, the interferences resulting from these constituents are cancelled by subjecting the refinery gas to a second contact period or blank run and subtracting the absorbance values obtained for the second extraction period from those obtained for the first extraction period. The accuracy of the blank correction method for petroleum refinery gas containing some or all of the above mentioned interferences and possible traces of benzene and xylene is exhibited by comparing the result of 10.1 p.p.m. of carbonyl sulfide with a value of 10.7 p.p.m., which was obtained by applying the same analytical technique to the propane-propylene fraction of the refinery gas in which the carbonyl sulfide was isolated from other interfering components by a low temperature fractional distillation (Figure 6).

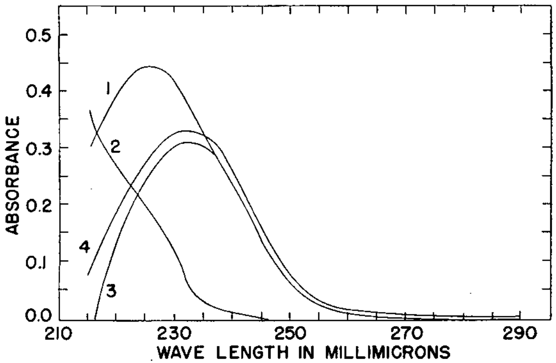


Figure 6. Ultraviolet spectrogram of unfractionated petroleum refinery gas sample and propane-propylene cut

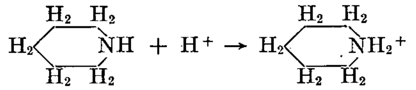
Unfractionated sample:

1. First 80-minute extraction
2. Second 80-minute extraction
3. Curve 1 — Curve 2, 10.1 p.p.m. carbonyl sulfide by volume

Fractionated sample:

4. Propane-propylene cut, 10.7 p.p.m. carbonyl sulfide by volume

The necessity for maintaining a careful pH control (11.0 to 11.4) of the aqueous piperidine extracting solution is shown in Figure 7. The addition of acid to the piperidine solution results in the formation of piperidinium ion which is spectrophotometrically apparent by a decrease in the absorption of the piperidine solution at wave lengths below 240 mμ. Further addition of acid does not affect the absorption properties of the solution once ionization is complete (curves 3 and 4, Figure 7). Carbonyl sulfide does not react with an aqueous solution containing completely ionized piperidine, as indicated by lack of absorption throughout the 215 to 290 mμ wave-length interval. It is also observed that the addition of sodium hydroxide has the opposite effect of acid addition and results in an increase in the absorption of the aqueous piperidine solution below 240 mμ. A solution buffered with sodium hydroxide was used with fair results. The absorption at 260 mμ, exhibited by all curves, is attributed to



$$C_5H_{10}NH + H^+ \rightarrow C_5H_{10}NH_2^+$$

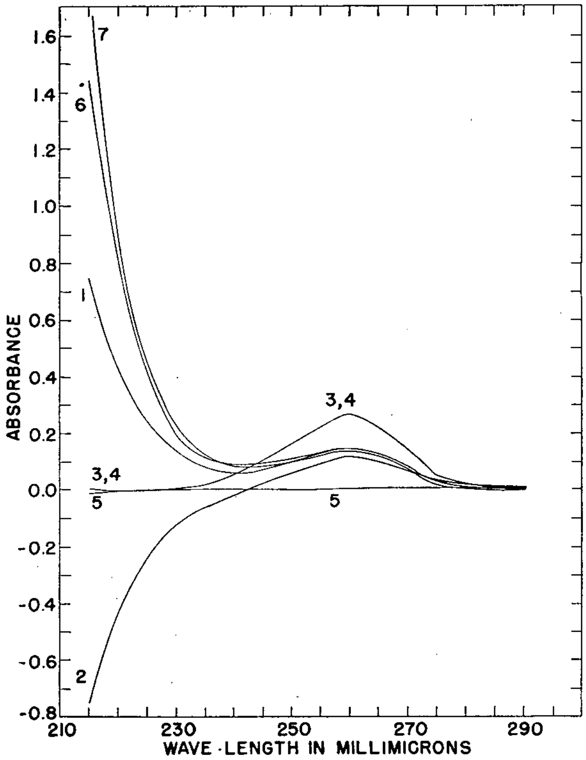


Figure 7. Effect of acidic and basic constituents on aqueous piperidine

1. Aqueous piperidine *vs.* distilled water
2. 0.1 ml. of 12*N* hydrochloric acid per 100 ml. of aqueous piperidine *vs.* aqueous piperidine
3. 0.1 ml. of 12*N* hydrochloric acid per 100 ml. of aqueous piperidine *vs.* distilled water
4. 0.4 ml. of 12*N* hydrochloric acid per 100 ml. of aqueous piperidine *vs.* distilled water
5. Carbonyl sulfide–propylene blend in completely ionized piperidine extraction solution
6. 0.054 gram of sodium hydroxide per 100 ml. of aqueous piperidine *vs.* distilled water
7. 0.11 gram of sodium hydroxide per 100 ml. of aqueous piperidine *vs.* distilled water

Table VI. Determination of Carbonyl Sulfide in a Propylene Synthetic Mix Containing Interfering Gases

Interfering Gas	Carbonyl Sulfide, P.P.M.	
	Present	Found
Hydrogen sulfide, 2520 p.p.m. plus methyl mercaptan, 149 p.p.m.	19.6	17.4
Carbon disulfide, 50.7 p.p.m.	18.4	19.3
Carbon dioxide, 176 p.p.m.	15.7	17.1
Carbon dioxide, 524 p.p.m.	13.7	14.2

Table VII. Determination of Carbonyl Sulfide in Petroleum Refinery Gas

Sample	Carbonyl Sulfide, P.P.M.
1	7.1
	7.4
2	10.1
	10.7
	10.9
3	13.0
	13.3
	13.3

the ionized form of piperidine. The negative values shown in curve 2, Figure 7, indicate that less light is transmitted by the unacidified aqueous piperidine than by the acidified aqueous piperidine at wave lengths lower than 240 mμ.

Accuracy and Applicability. In Table VI the accuracy of the determination is exhibited for various synthetic mixtures. The gases were prepared by adding accurately measured volumes of carbonyl sulfide to the evacuated calibrated sampling flask and diluting with hydrogen sulfide, methyl mercaptan, carbon dioxide, carbon disulfide, and propylene.

In the one instance where carbon disulfide was the only interfering gas, it was not necessary to use any scrubbers because the interference was corrected by application of a blank run.

The applicability of the method for petroleum refinery gas samples representing the combined feeds from thermal and catalytic cracking units is illustrated in Table VII.

Additional justification for the method was obtained by adding 9.0 p.p.m. of pure carbonyl sulfide to the sample of refinery gas containing 7.3 p.p.m. and subsequently finding a total of 15.7 p.p.m.

The accuracy of the method in the absence of interfering components is 1 p.p.m. within the range of 1 to 50 p.p.m. (Table I). The same accuracy holds for gas samples containing interfering components such as carbon disulfide, thiophene, and 1,3-butadiene that can be corrected by application of a blank run. When the gas sample contains interfering components requiring the use of scrubbers containing Shaw's reagent and 30% sodium hydroxide, as much as 10% hydrolysis of carbonyl sulfide occurs within the concentration range of 1 to 50 p.p.m

ACKNOWLEDGMENT

The authors wish to thank Kenton Atwood of the Girdler Co. for his advice and suggestions during the course of this study.

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Received for review May 26, 1955. Accepted January 16, 1956.

Spectrographic Determination of Silica

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The application of spectrographic analysis to the quantitative determination of major constituents of inorganic substances has had limited success. The specific purpose of this investigation was to develop a spectrographic method for the quantitative determination of silica in high concentrations by using germanium metal as an internal standard. The germanium serves as an excellent buffer and produces a very stable arc in which the sample is completely and homogeneously volatilized. Known mixtures of silica and calcium carbonate were used as standard samples for the preparation of the analytical curves. The line absorbance values of the line pair used permit the use of the more rapid line absorbance ratio method of graphing results without sacrifice of accuracy. The procedure was tested with analyzed samples of siliceous shales, cherts, and other rocks of high silica content. The standard deviation was 0.94% for the range from 0 to 20% silica, and 1.72% for the range from 20 to 70% silica.

THE use of spectrographic methods has been confined mainly to two general types of analysis: (1) the determination of the concentrations of minor constituents in a sample, and (2) the identification of the constituents of small samples. Although the majority of the spectrographic analyses deal with the determination of minor rather than major constituents, some methods for major constituents have been reported in the literature (*1–4, 6–11, 13*). In most of these a buffer material is used as an internal standard for line comparison purposes, and carbon is added to render the mixture conducting as well as to obtain smoother burning of the sample.

Nickel oxide was used as a buffer by Oshry, Ballard, and Schrenk (*8*), and cupric oxide by Jaycox (*6*); a mixture of barium nitrate and ammonium sulfate was reported by Fitz and Murray (*1*); beryllium carbonate in a mixture with sodium chloride was used by Kvalheim (*7*). Herdle and Wolthorn (*4*) used silica, the major constituent of the sample, as the internal standard in analyzing silica refractories for the other elements, and then determined silica by difference. Gamble and Kling (*2*) used lithium carbonate as a flux and diluent in the determination of silica in petroleum ash. Steinberg and Belic (*11*) described a method for the quantitative spectrographic analysis of open-hearth slag samples by the use of the lime-silica ratio. Most of the methods reported indicated that it was necessary to use more than one spectral line for each element if the concentration range over which the determinations were being made exceeded 20%. The deviation in most instances was 4 to 10% if the concentration of the constituent being determined was greater than 20%.

This paper describes a spectrographic technique for the determination of silica in powdered samples of rocks, using germanium metal as an internal standard and carbon powder as a diluent. Strock (*12*) suggested the use of germanium metal in the matrix in the spectrographic determination of a number of major constituents in geological samples. The method herein described is now being extended to cover all the normal constituents of rocks to see if it is possible to obtain a complete analysis on a single sample. The suggested method, which was tested on limestones, siliceous shales, cherts, and other rocks of high silica content, requires the use of only 10 mg. of sample and may be employed for the determination of both major and minor constituents.

Strock (*12*) cited the following reasons for selecting germanium metal as a matrix: the high regularity of concentration calibration curves for minor constituents in germanium metal powder synthetic standards; the marked suppression of fractional distillation from a direct current arc rich in metallic germanium metal; the similar temperature range of the liquid state of many metals and that of liquid germanium (thus affording the possibility of volatilizing many elements from a homogeneous metallic solution); and the fact that the ionization potential of germanium is above that of most other metals, lying between them and the carbon of the electrodes, and thus reducing the necessity of the sample elements providing the conducting particles and competing with each other in the process.

EQUIPMENT

A Baird Associates' 3-meter grating type spectrograph was used, with a 15,000-line-per-inch grating having a uniform dispersion of 5.56 A. per millimeter in the first order. A six-step sector with an exposure ratio of 2 to 1 was rotated in front of the slit at 1400 r.p.m. Excitation was obtained from a controlled multisource power unit set for 50-volt 8-ampere direct current arc. The instrument was equipped with a 100-micron slit which was illuminated with approximately parallel light by the use of a quartz cylindrical condensing lens. The spectrum was recorded on Eastman spectrographic plates Type II-O, which were developed for 3.5 minutes with continuous agitation in a constant temperature tank with Eastman D-19 developer. Spectral line absorbances were determined by the use of a Leeds & Northrup recording microphotometer, which gives direct readings of the absorbances. The electrodes were prepared from regular spectrographic carbon rods 12 by $^1/_4$ inches, supplied by the National Carbon Co., New York. The upper or counter electrode was shaped with a flat end, and the lower or supporting electrode was a center-post crater type having a crater $^1/_4$ inch deep. A machine similar to that described by Oshry, Ballard, and Schrenk (*8*) was used to shape and bore the electrodes.

DEVELOPMENT OF METHOD

Various spectral lines of silicon and germanium were studied in order to determine a homologous pair which would give maximum reproducibility, would be free from interference, and would be closely spaced on the plate. Silicon line 2881 A. and germanium line 2556 A. were found to be a suitable pair which produced satisfactory absorbance values.

Synthetic samples of the oxides of the most common elements in silicate and mineral rocks, such as iron, aluminum, magnesium, and calcium, were mixed in various proportions. These samples were then used to prepare mixtures in which the amount of silica was kept constant and the other elements varied in order to determine the effect of these elements on the silicon lines. Other mixtures were prepared in which the concentration of silica was varied to determine its effect on the lines of the other elements. It was found that the lines selected were free from interference.

One-gram standard samples containing only calcium carbonate and silica were prepared for the development of the analytical curves by varying the weight per cent of silica from 0 to 100% and inversely varying the amount of calcium carbonate. This mixture was selected to save time involved in the weighing of several components, because the primary interest was the determination of silica.

Ten milligrams of the standard samples were mixed with various amounts of the germanium metal and powdered carbon to determine a suitable mixture for producing satisfactory line absorbance values of the lines chosen. A mixture containing 10 mg. of standard sample, 90 mg. of germanium metal, and 800 mg. of powdered carbon was selected, because it produced satisfactory line absorbance values and permitted the use of sector steps of the spectral lines in which the plate background density was at a minimum.

PROCEDURE

Preparation of Samples. Reagent grade silicic acid and calcium carbonate were used for the preparation of the standard samples. The silicic acid was ignited at 900° C. for 4 hours to ensure complete dehydration. The calcium carbonate was dried at 105° C. for 1 hour. The components were weighed accurately in the required proportions for the preparation of 1-gram samples. Spectrographically pure germanium metal was used for the internal standard. The powdered carbon used as a diluent was obtained during the shaping of the carbon electrodes and was purified by treatment with hot concentrated hydrochloric acid to remove any trace contaminants.

The rock samples used to check the analytical curves were ground to pass a 300-mesh sieve and were analyzed gravimetrically for silica by procedures outlined by Hillebrand and Lundell (*5*).

The required weights of sample, germanium, and carbon were thoroughly mixed in an agate mortar. A metal funnel designed to prevent any loss of sample was used to transfer 75 mg. of this mixture to the center-post crater-type carbon electrode. The material was then packed with a special metal tamper of correct dimensions so that it fitted the crater and center post. This ensured uniform packing of the sample without loss, and reduced the possibility that any portion might be blown out of the crater when the arc was turned on. Duplicate samples were burned to check reproducibility and to facilitate line absorbance measurements. The mixture burned in each electrode contained only 0.833 mg. of the original sample. This small amount caused negligible interference between the various elements present and made it possible to analyze samples of widely varying composition with consistent results. The interferences are rendered negligible because the sample and germanium are diluted with carbon in the ratio of 1 to 8 instead of the usual ratio of 1 to 2. At such low dilutions the atomic population of the sample in the arc gases is reduced to such a level that the excitation energy-collision exchange processes are no longer important in quantitative measurements.

Preparation of Spectrograms. The electrodes were placed in the holders in the arc stand with the lower electrode at a positive potential with respect to the upper electrode. The electrodes were aligned on the optic axis and were permitted to touch during a preheating period of 10 seconds. The shutter was opened, the arc turned on, and at the end of the preheating period, the electrodes were separated by a distance of 5 mm. and kept at this distance until the sample was completely burned, which required approximately 300 seconds. The plates were then developed and air-dried.

The silicon line employed permitted the use of a sector step which was relatively free from plate background density and gave satisfactory line absorbance values. The developed plates were read with the microphotometer, using lines Si-2881 and Ge-2556 to cover the entire range from 0 to 70% silica.

RESULTS

The analytical curves shown in Figures 1 and 2, which were prepared from samples containing known amounts of silica, show the variation of the weight per cent of silica with the ratio of the absorbances of the Si-2881 and the Ge-2556 lines. This method of graphing, which has not been widely used in the past, has certain advantages for this particular set of data over the usual method in which the radiant power (intensity) ratio of the lines is plotted against the weight per cent of the constituent being determined on log-log paper. One of these advantages is the simplification of calculations due to the fact that the ratio is found by simple division of the direct microphotometer readings and only one graph is needed. Another advantage is that the value of the ratio of the absorbances of the lines is not affected greatly by normal variations in excitation conditions nor by slight variations in development procedure, because the two lines used constitute a homologous pair. The third advantage is that use of the absorbance ratio in place of the radiant power ratio gives satisfactory results over a wider range of percentages of silica. Part of the reason for this third advantage resides in the fact that a section of the normally nonlinear portion of the gamma curve can be utilized for calibration and measurements. This advantage is not without sacrifice of some convenience because each new batch of spectrographic plates requires key check recalibration measurements over a wide range of silica values, and possible correction of the slope of the calibration line. The recalibration is necessary because the procedure described here does not take into account differences in photographic emulsions. In many cases it will be satisfactory to use standard samples that have been determined previously by this method to make these calibration corrections.

Two calibration curves are used because of an apparent change in slope between 20 and 25% silica. This change is apparently due to the poorer microphotometry in the lower ranges of silica concentration. The best straight lines were fitted to the points in Figures 1 and 2 by the method of least squares. The values of the ratios used in Figure 1 ranged from 0.174 to 2.020, with an average value of 0.992 and a standard deviation of 0.023. In Figure 2, these values ranged from 1.60 to 6.19 with an average value of 3.34 and a standard deviation of 0.079.

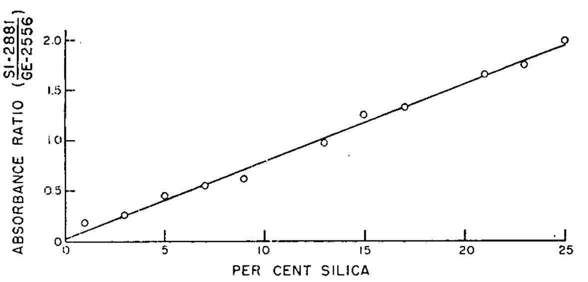

Figure 1. Calibration curve, 0 to 25% silica

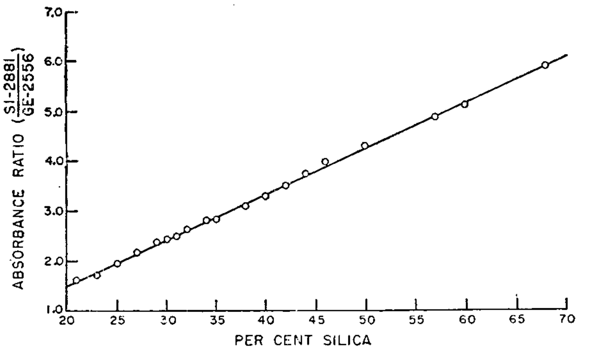

Figure 2. Calibration curve, 20 to 70% silica

Table I. Spectrographic and Chemical Analyses Using Line Pair Si-2881 and Ge-2556 for Limestone Samples in Range 0 to 20% Silica

	Absorbance			Weight % Silica		
Sample	Si-2881	Ge-2556	Ratio Si/Ge	Spectrographic	Chemical	Diff.
1	0.014	0.117	0.120	1.2	0.4	0.8
	0.019	0.123	0.154	1.6		1.2
2	0.029	0.160	0.181	2.0	1.2	0.8
	0.022	0.110	0.200	2.3		1.1
3	0.072	0.128	0.562	7.1	5.5	1.6
	0.063	0.122	0.516	6.5		1.0
4	0.045	0.126	0.357	4.4	6.1	1.7
	0.041	0.113	0.363	4.4		1.7
5	0.053	0.110	0.482	6.1	6.3	0.2
	0.045	0.100	0.450	5.6		0.7
6	0.145	0.145	1.000	12.8	13.1	0.3
	0.090	0.110	0.818	10.4		2.7
7[a]	0.112	0.102	1.098	14.1	14.1	0.0
	0.108	0.102	1.059	13.5		0.6
8	0.175	0.148	1.182	15.2	17.0	1.8
	0.188	0.135	1.393	17.9		0.9
Std. dev.	0.0147	0.0161	0.0724	0.94	Av.	1.07

[a] National Bureau of Standards standard sample 1a, argillaceous limestone.

The results of the analysis of a number of rock samples are given in Tables I and II. These tables show that values for samples which contain up to 70% of silica are satisfactory by the proposed method. For samples below 20% silica, the standard deviation of the spectrographic results is 0.94, and the average difference between the chemical and spectrographic determinations is 1.07%. For samples between 20 and 70% silica, the standard deviation of the spectrographic results is 1.74 and the average difference between the chemical and spectrographic determinations is 1.16%. The poor results for percentages below 13 are probably due to the poorer microphotometry in the use of a single line pair for the entire range.

DISCUSSION

The amount of sample required in the proposed procedure is only 10 mg., and may be of the order of about 1 mg. The germanium serves not only as an internal standard but as an excellent buffer for producing a very stable arc with complete volatilization and the cost of the small quantity required in the mixture is nominal.

The use of one spectral line for the determination of silica over the entire range up to 70% reduces the time required for microphotometer operations.

It is essential that the silica used in preparing the standard samples be completely dehydrated in order to ensure consistent

Table II. Spectrographic and Chemical Analyses Using Line Pair Si-2881 and Ge-2556 for Samples in Range 20 to 70% Silica

	Absorbance			Weight % Silica		
Sample[a]	Si-2881	Ge-2556	Ratio Si/Ge	Spectrographic	Chemical	Diff.
9	0.202	0.121	1.669	21.6	21.9	0.3
	0.266	0.147	1.810	23.2		1.3
10	0.345	0.166	2.078	26.2	27.7	1.5
	0.377	0.170	2.218	27.7		0
11	0.446	0.156	2.859	34.7	31.9	2.8
	0.452	0.173	2.613	32.0		0.1
12	0.425	0.130	3.269	39.1	38.8	0.3
	0.376	0.117	3.214	38.5		0.3
13	0.429	0.136	3.154	37.9	38.9	1.0
	0.540	0.172	3.140	37.7		1.2
14	0.491	0.128	3.840	45.2	47.5	2.3
	0.661	0.151	4.377	51.2		3.7
15	0.590	0.137	4.307	50.5	52.1	1.6
	0.560	0.128	4.375	51.1		1.0
16	0.675	0.135	5.000	58.1	57.5	0.6
	0.685	0.136	5.037	58.3		0.8
17	0.762	0.126	6.048	69.2	67.5	1.7
	0.742	0.126	5.889	67.1		0.4
Std. dev.	0.0528	0.0130	0.153	1.74	Av.	1.16

[a] Sample 9 is limestone, samples 10 to 16 are siliceous shales, and sample 17 is chert. Sample 16 is a mixture of equal parts of samples 14 and 17.

results. The silica–calcium carbonate mixtures should be dried at 105° C. before weighing for mixing with the germanium and carbon to expel moisture absorbed during the original mixing operations.

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Received for review April 1, 1955. Accepted February 6, 1956. Taken in part from the thesis submitted by John W. Anthony to the Graduate School of Southern Methodist University in partial fulfillment of the requirements for the degree of master of science. A cooperative project between the Department of Chemistry, Southern Methodist University, Dallas, Tex., and the Magnolia Petroleum Co., Field Research Laboratories, Dallas, Tex.

Effect of Aging Solutions of Barium Chloride on Particle Size of Barium Sulfate

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The aging of barium chloride solutions which were used to precipitate barium sulfate was found to cause an increase in the particle size of the precipitate. Filtration of a freshly prepared solution of barium chloride through a fine sintered-glass or porcelain filtering crucible produced the same effect as aging. The particle size of the precipitate seems to be a function of the number of nuclei which are available as starting points for crystallization. The origin of the nuclei was not established with certainty, but considerable evidence supports the theory that aggregates of incompletely dissolved barium chloride in the fresh solutions may serve as nuclei for starting crystals of barium sulfate.

STUDIES on the coagulation of barium sulfate (*5*) by use of minute quantities of agar led to the observation that marked differences in the particle size of precipitates were caused by the age of the solutions of barium chloride which were used to precipitate barium sulfate. An abstract (*3*) of these studies was published in 1949. Similar observations have been reported by Fischer and Rhinehammer (*7*). The significance of the age of precipitating solutions seems to have been overlooked by the many investigators (*8*) who have studied the factors affecting the particle size of analytical precipitates. However, Bancroft (*1*) stated that, in his opinion, the number of nuclei present in solution is more important than the extent of supersaturation as proposed by von Weimarn (*8*).

Benedetti-Pichler (*2*) in an exchange of correspondence with one of the authors (*4*) reported that he found an aging effect which disappeared on recrystallizing the barium chloride. However, he mixed the sulfate and barium solutions simultaneously near boiling temperatures, whereas the authors added the barium chloride solution from a pipet with a constant delivery time at room temperature. Benedetti-Pichler reported barium sulfate crystals of 5 to 6 microns from purified barium chloride. This size is approximately the same as was obtained in this laboratory from fresh solutions of barium chloride. This is shown in *A* of Figure 1, in which the scale division of 18 microns is close to 3 times the diameter of the average crystal. The effect of aging the barium chloride on the size of the barium sulfate crystals is shown in *B*, *C*, *D*, and *E* of Figure 1. The effect of filtering a fresh solution of barium chloride through a fine (2- to 5-micron)

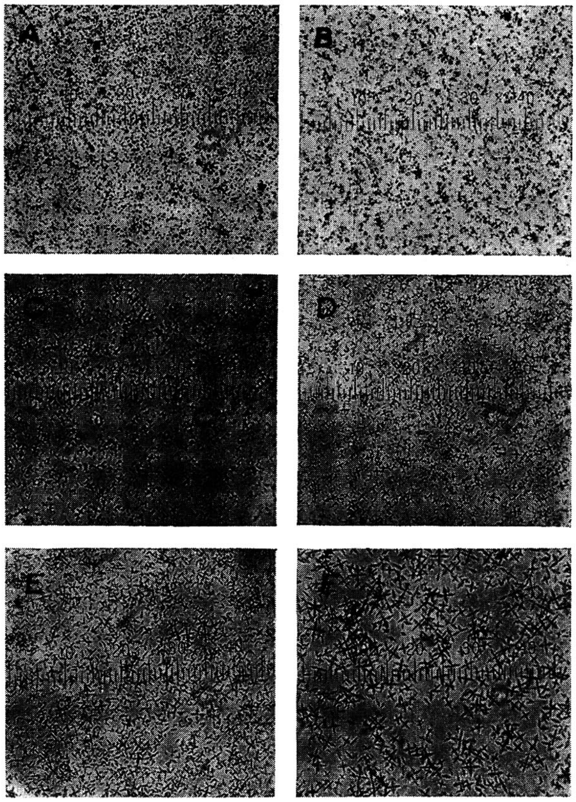

Figure 1. Crystals of barium sulfate

1 scale division = 0.018 mm. Crystals formed under identical conditions except for treatment of 5% barium chloride dihydrate solutions.

A. Fresh solution
B. Aged 2 hours
C. Aged 7 hours
D. Aged 24 hours
E. Aged 1 week
F. Fresh solution filtered through fine sintered-glass filter

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Selas filtering crucible is shown in *F* of Figure 1. Fully aged or filtered barium chloride solutions produced crystals from 7 to 10 times as large as those obtained from fresh barium chloride solutions. Filtration of barium chloride solutions through Blue Ribbon S. and S. filter paper had practically no effect on the crystal size of the barium sulfate.

The effect of aging solutions of barium chloride was observed, for the most part, by measuring the rate of settling of suspensions of barium sulfate. The sedimentation measurements were made at temperatures constant within 1° C. The apparatus used was similar to the one designed by Calbeck and Harner (*6*) and is shown in Figure 2. The series of curves shown in Figure 3 was obtained by this method. The differences in settling rates after 1 week are probably not significant, because they are close to the precision of the method of measurement.

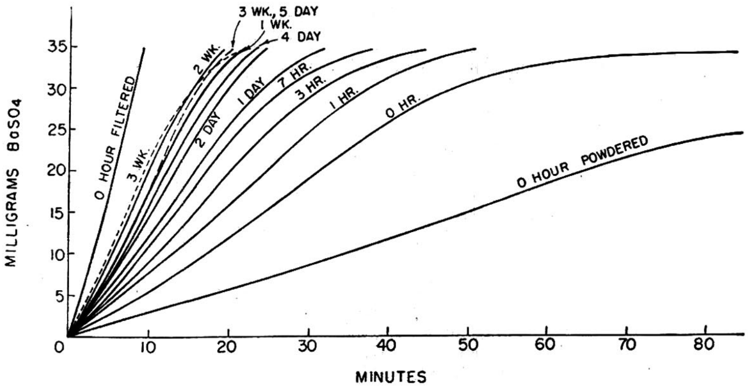

Figure 3. Settling rates of barium sulfate from 5% solutions of barium chloride dihydrate

EXPERIMENTAL

Procedure. A solution of sodium sulfate which had been aged for several weeks was used to supply the sulfate ions for the precipitation of barium sulfate. The concentration of the sodium sulfate was adjusted so that 25 ml. gave 0.100 gram of barium sulfate. In all precipitations 25 ml. of the sodium sulfate solution was delivered from the same pipet into a 300-ml. Erlenmeyer flask, 1 ml. of 1*N* hydrochloric acid was added, and then the solution was diluted with 200 ml. of distilled water. The contents of the flask were mixed thoroughly and permitted to stand for a few minutes until the agitation from mixing had subsided. Then 25 ml. of 4 or 5% barium chloride solution was delivered at room temperature and at the maximum rate of flow from a pipet which was held vertically approximately 1 inch above the surface of the sulfate solution. The same pipet, which had a delivery time of 35 seconds, was used in all precipitations. After addition of the barium chloride, the contents of the flask were thoroughly shaken and permitted to stand for 24 hours at room temperature before measuring the settling time of the precipitate. A few degrees difference in temperature at the time of precipitation was found to cause a negligible difference in the size of the crystals. The rate of settling, however, was sensitive to changes in temperature, hence settling rates were considered valid only if the temperature remained constant within 1° C. during the measurement.

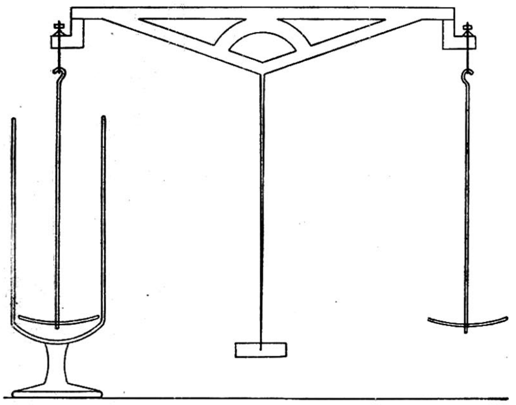

Figure 2. Apparatus used to measure settling rates of barium sulfate

The rate of settling of the precipitate was determined by observing, with a stop watch, the time required for 5-mg. increments (under water weight) of barium sulfate to settle on a watch glass attached by a glass rod to the left arm of a damped chain-weight balance. At the start of the measurement the precipitate was dispersed by moving the watch glass up and down in the solution with the container in place under the arm of the balance. The watch glass was then quickly hooked into the stirrup of the balance and the chain was adjusted until a steady settling rate was observed. After a little practice the position of the chain at the start of the observations could be checked within 5 mg. of the same starting weight. Small additions of weight were made by rolling down the chain as the barium sulfate settled on the watch glass. The chain weight was increased as soon as the pointer was displaced approximately 0.5 division from the

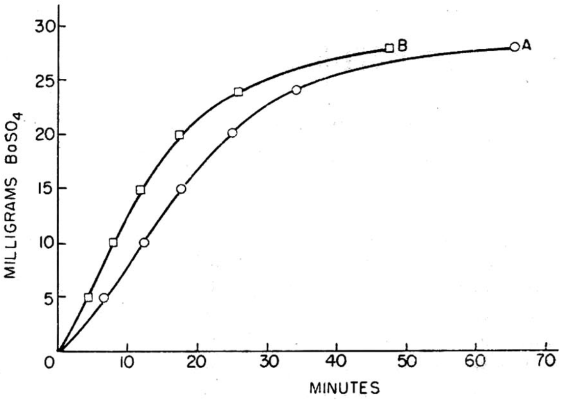

Figure 4. Settling rates of barium sulfate

From barium chloride dihydrate
A. Fresh solution, 4%
B. Fresh solution of recrystallized barium chloride

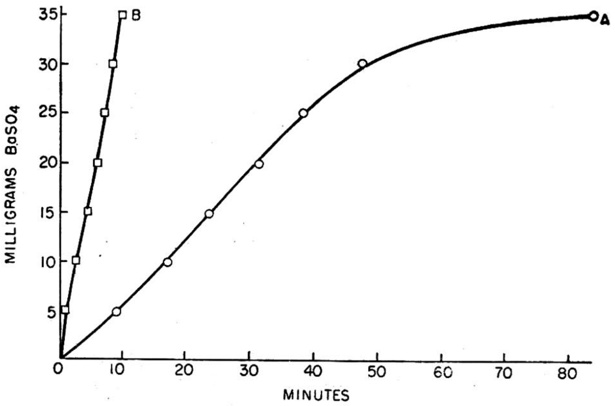

Figure 5. Settling rates of barium sulfate

From barium chloride dihydrate
A. Fresh solution, 5%
B. Fresh solution filtered through fine (2- to 5- micron) sintered-glass filter

central position on the pointer scale. Observations were made to show the settling time for 25 to 35 mg. (under water weight) of barium sulfate. Various factors which might alter the size of the crystals of barium sulfate were investigated.

Distilled Water. Ordinary distilled water, double-distilled water from thoroughly steamed apparatus, and water distilled from a solution of barium chloride were used to prepare solutions of barium chloride. Only a slight increase in the particle size of barium sulfate was observed in precipitations made from solutions prepared from highly purified water. However, double-distilled water was used in all subsequent experiments.

Recrystallized Barium Chloride. Reagent grade barium chloride was dissolved in hot water and filtered through a fine sintered-glass filtering crucible. The recrystallized barium chloride was used to prepare a solution of barium chloride which was used immediately to precipitate barium sulfate. A slight increase in crystal size was observed as shown in Figure 4, but the increase in particle size was not comparable with that obtained from aged or filtered barium chloride solutions as shown in Figure 5.

Concentration of Barium Chloride. The concentration of barium chloride in the solutions which were allowed to age was either 40 or 50 grams of barium chloride dihydrate per liter. No difference in the aging properties of the 4 and the 5% solutions was observed. However, when the concentration was reduced to 1%, the aging effect appeared complete in 24 hours as shown in Figure 6.

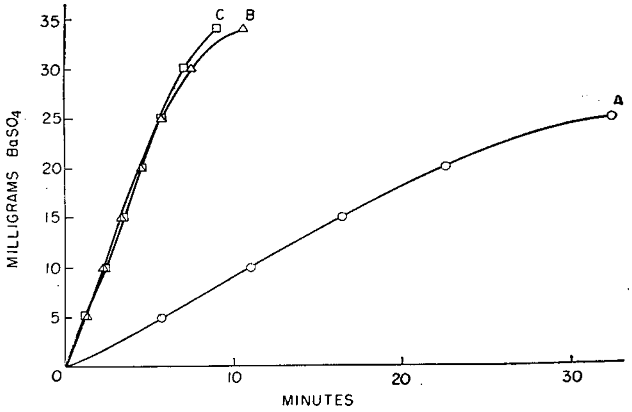

Figure 6. Settling rates of barium sulfate

From 1% barium chloride dihydrate

A. Fresh solution
B. Aged 24 hours
C. Fresh solution filtered through fine sintered-glass filter

Dehydrated Barium Chloride. Much smaller crystals of barium sulfate were obtained from fresh solutions of the dehydrated salt than from similar solutions of the hydrated salt. Aging the solutions of the dehydrated salt caused the usual increase in the particle size of the barium sulfate. A number of experiments were made to determine whether a filtered solution of barium chloride which gave large crystals of barium sulfate would continue to give large crystals if the solution was evaporated to dryness in a platinum dish, dehydrated, and then dissolved to prepare a fresh solution. The formation of even finer crystals of barium sulfate was observed from such solutions. The large crystals reappeared on filtering through sintered glass, but after a second evaporation and dehydration the fine crystals again formed when a fresh solution was used to precipitate barium sulfate. These results are shown in Figure 7.

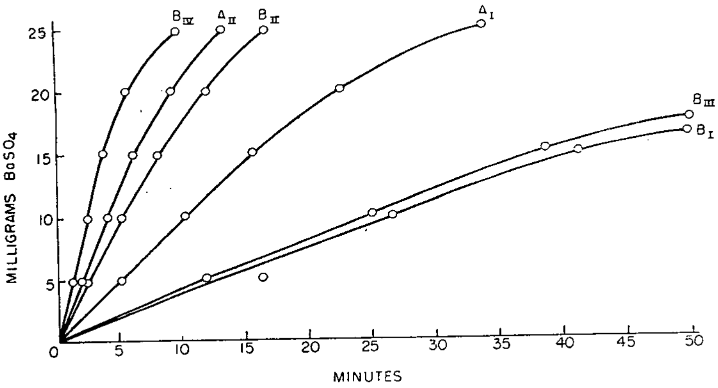

Figure 7. Settling rates of barium sulfate

A_I. Fresh 4% solution of recrystallized barium chloride dihydrate
A_{II}. From solution A_I after filtering
B_I. Fresh solution of anhydrous barium chloride
B_{II}. From solution B_I after filtering
B_{III}. From B_{II} after filtration, evaporation, and dehydration
B_{IV}. From solution B_{III} after aging 72 hours

Effect of Temperature on Aging. If barium chloride is dissolved in boiling water, then allowed to cool to room temperature and used to precipitate barium sulfate, the precipitated particles are larger than crystals obtained from solutions of barium chloride prepared at room temperature and aged for the same length of time. The effect of temperature on the rate of aging is indicated in Figure 8, which shows that somewhat larger crystals of barium sulfate were obtained from a solution of barium chloride which had been aged for approximately 6 days at a temperature about 20° C. higher than a second solution, which was aged in a refrigerator.

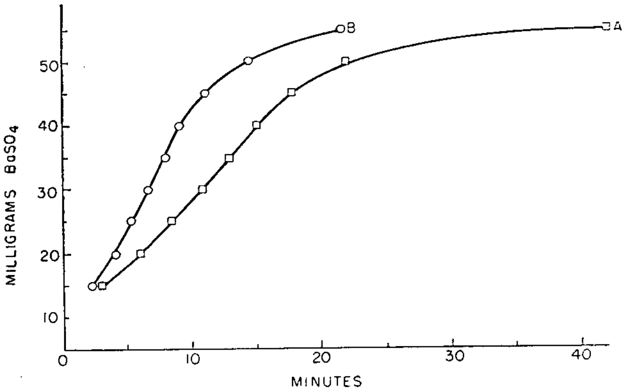

Figure 8. Settling rates of barium sulfate

From barium chloride dihydrate

A. Aged 6 days at 6° C.
B. Aged 6 days at room temperature

Powdering the Barium Chloride. A fresh solution of barium chloride was prepared from crystals ground to a fine powder in an agate mortar. Extremely fine crystals of barium sulfate were obtained from the ground hydrated salt. They were similar in size to those obtained from the dehydrated salt. The effect of powdering the barium chloride is shown in Figures 3 and 9.

Large Crystals of Barium Chloride. Large nearly perfect crystals of barium chloride were prepared by the slow growth of a few crystals suspended for several weeks in a slightly supersaturated solution of barium chloride. A single crystal weighing 2.720 grams was dissolved in 68 ml. of double-distilled water. A 25-ml. portion of the fresh solution was used to precipitate barium sulfate in the usual manner. For the first time large crystals of barium sulfate were obtained from a fresh unfiltered solution of barium chloride. The settling rate is shown in Figure 9, curve *A*. A slight increase in crystal size was observed after aging 48 hours as shown in Figure 9, curve *B*.

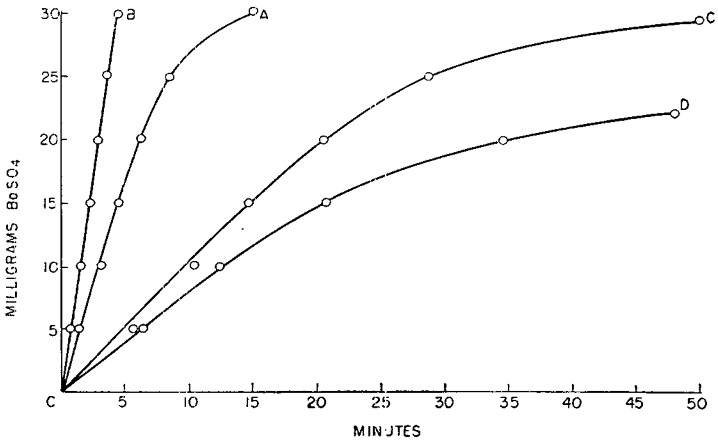

Figure 9. Settling rates of barium sulfate

A. From fresh solution of large single crystal of barium chloride dihydrate
B. From solution *A* after aging 48 hours
C. From fresh solution of crushed large crystals of barium chloride dihydrate
D. From fresh solution of dehydrated large crystals of barium chloride

Several of the large crystals of barium chloride were crushed to a powder in an agate mortar and used to prepare a solution of barium chloride. The solution of powdered barium chloride produced fine crystals of barium sulfate as shown in Figure 9, curve *C*. The dehydrated salt from the large crystals also produced small crystals of barium sulfate, Figure 9, curve *D*.

Time for Precipitate to Appear. It was observed that a shorter time was required for the beginning of precipitation when a fresh solution was used than when an aged solution of barium chloride was used. An average of 0.6 second from the time the fresh solution entered the sulfate solution was required for visible precipitation, whereas 11.5 seconds was the average time for visible precipitation when an aged solution was used.

DISCUSSION

The abundance or scarcity of nuclei which serve as starting points for crystallization seems to be the most reasonable explanation for the observed facts. If many nuclei are present the crystals of barium sulfate will be small, but if relatively few nuclei are available the crystals will grow to a greater size. The fact that large crystals are always obtained after aging or filtering the precipitant suggests that attention should be given this point in preparing solutions for analytical precipitations.

The effect of aging the precipitant on the particle size of precipitates was observed in solutions other than barium chloride. Solutions of sodium sulfate showed a similar, although more rapid, aging effect when used in the reverse precipitation of barium sulfate. The aging of sodium sulfate solutions appeared to be complete in 24 hours. Calcium chloride solutions gave larger crystals of calcium oxalate after 24 hours of aging. Aged solutions of barium chloride gave larger crystals of barium chromate than fresh solutions.

Experiments were made to test various theories regarding the source of the crystal nuclei. Dust particles, traces of barium sulfate, dissolved air, and impurities in the distilled water and the barium chloride were ruled out as accounting for the aging effect. Traces of barium sulfate seemed a possible explanation until it was found that barium chloride in a filtered solution gave large crystals, but would again produce small crystals if evaporated to dryness, redissolved, and used while fresh. This experiment was repeated several times with the same solution (Figure 7). It was concluded that traces of barium sulfate must have been removed, if its removal was the cause of the formation of large crystals in the filtered solution. The reappearance of small crystals after each evaporation and preparation of a fresh solution indicates that the nuclei must come from the barium chloride itself and not from traces of barium sulfate. The only unfiltered fresh solutions of barium chloride which gave large crystals were obtained from solutions of large single crystals of barium chloride. Powdering or dehydrating these crystals again produced a fine precipitate of barium sulfate.

An explanation which seems consistent with the observed facts, but which admittedly is not proved, is that a fresh unfiltered solution contains aggregates of barium chloride which have not yet dispersed into barium and chloride ions. These aggregates may serve as nuclei for the starting of barium sulfate crystals. The adsorption of air on the surface of fine crystals of barium chloride may be a factor in causing the residues of crystals to persist as aggregates for a considerable time. The remarkable fact that large crystals of barium chloride gave large crystals of barium sulfate may be due to the absence of crystal residues to serve as nuclei for crystallization. The formation of fine crystals of barium sulfate when the large crystals of barium chloride were powdered or dehydrated is consistent with the theory of crystal residues and would appear to rule out impurities in the salt as an explanation of the observed facts. The effect of filtration through sintered glass removes many nuclei, possibly by dispersion of the aggregates rather than by adsorption, because there was no indication of saturation of the glass filter after a liter of barium chloride solution had been filtered.

SUMMARY

A marked increase in the particle size of crystals of barium sulfate was observed when aged or filtered solutions of barium chloride were used in precipitations. The particle size was assumed to be caused by the abundance or scarcity of crystal nuclei in the barium chloride solution.

Numerous experiments were performed in an attempt to find the source of the crystal nuclei. The observed facts suggest, but do not prove, that aggregates of incompletely dissolved barium chloride may serve as nuclei for the starting of crystals of barium sulfate.

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Received for review August 24, 1955 Accepted January 3, 1956.

Determination of Triphosphate in Commercial Triphosphate and Detergents Built with Triphosphate

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Tris(ethylenediamine) cobalt(III) ion [$Co(en)_3^{+++}$] has been shown to be a precipitant for triphosphate ($P_3O_{10}^{-5}$) ion in an acid solution. A tendency for pyrophosphate to coprecipitate was reported. Compensation for such interference is made in this method by use of a calibration curve and carefully controlled precipitation conditions. Based on analyses of mixtures containing known amounts of triphosphate, the method shows a standard deviation of 0.8% absolute. The reproducibility of the method was determined by carrying out replicate determinations on commercial triphosphate and triphosphate built anionic detergents. A value of 0.5% absolute was obtained. The triphosphate determination can be run in 2 hours. The method is applicable to commercial triphosphate and triphosphate built detergent samples. Appreciable amounts of phosphate glass interfere with the determination, but the method gives warning when such interference takes place. Methods are also recommended for determination of orthophosphate, trimetaphosphate, and total phosphate.

WITH the increased use of detergents containing sodium triphosphate as a builder, the need for a specific method of determining the triphosphate content of commercial triphosphate and finished products containing triphosphate has arisen. In both cases other phosphates (ortho-, pyro-, and sometimes trimetaphosphate) are present with the triphosphate. As noted previously (*12*), a number of methods depending upon zinc precipitation have been used, but are not wholly satisfactory because the triphosphate content is determined indirectly and other ions interfere. Adequate compensation for the interferences has not been made in the methods. An x-ray method (*7*) is free of interference from other phosphates; however, it requires that all the material to be determined be crystalline and that materials having diffraction lines in the same positions as the measured phosphate lines be absent. A method involving the use of the isotope dilution technique (*12*) is specific for pyro- and triphosphate species. Equipment to make radiochemical measurements is required and the elapsed time for completing the determination is 1 to 3 days. Therefore, a need still exists for a reasonably rapid and accurate method of determining triphosphate in the presence of the other phosphates.

Hexamminecobalt(III) chloride has been suggested as a reagent in qualitative microchemical tests for pyrophosphate (*3*) and triphosphate (*9*). The amperometric titration of pyrophosphate with this reagent has been described (*6*).

Recently the precipitation of the phosphates with tris(ethylenediamine) cobalt(III) ion has been studied (*8*) and it was shown that maximum yields of crystals having the composition $Co(en)_3H_2P_3O_{10}.2H_2O$ and $Co(en)_3HP_2O_7$ were obtained at pH 3.5 and 6.5, respectively. These precipitations were carried out on the individual pure phosphates. At pH 3.5 pyrophosphate was not precipitated and at pH 6.5 triphosphate was not precipitated. When triphosphate was precipitated from a solution containing pyrophosphate, some of the pyrophosphate was coprecipitated. The freedom from interference of other phosphates, sulfate, and silicate indicated that it might be worth while to look for conditions which would minimize or compensate for the pyrophosphate interference. This possibility was investigated and the method which resulted is here reported.

MATERIALS

Tris(ethylenediamine) Cobalt(III) Chloride. This material was prepared by a published method (*2*). It was recrystallized, and dried at 110° C. just before use, because it is hygroscopic. The reagent solution consisted of 10.0 grams of freshly dried compound dissolved in 250 ml. of distilled water. One drop of toluene was added to inhibit mold growth.

Phosphates. SODIUM TRIPHOSPHATE. The commercial material was dissolved in water and the hexahydrate precipitated with alcohol. A total of four recrystallizations was carried out. The final product was air-dried and stored as sodium triphosphate hexahydrate ($Na_5P_3O_{10}.6H_2O$).

SODIUM PYROPHOSPHATE. Reagent grade tetrasodium pyrophosphate decahydrate ($Na_4P_2O_7.10H_2O$) was recrystallized and dried at 110° C. The product was stored in tightly stoppered bottles and weighed as the anhydrous salt.

SODIUM TRIMETAPHOSPHATE. The monohydrate was prepared from the commercial material by precipitating from a water solution with ethyl alcohol at 40° C. The material was air-dried and weighed as sodium trimetaphosphate monohydrate ($Na_3P_3O_9.H_2O$).

PHOSPHATE GLASS. Calgon, having an average degree of polymerization from 10 to 15, was used as received from Calgon, Inc., Pittsburgh, Pa. Quadrafos, having an average degree of polymerization of 4 to 5, was used as received from the Rumford Chemical Works, Rumford, R. I.

SODIUM TETRAPHOSPHATE. The sodium tetraphosphate was prepared as an 11% aqueous solution as described by Quimby (*11*).

Stock Buffer Solution, pH 3.6. An aqueous solution was made by dissolving 52.6 ml. of reagent grade glacial acetic acid and 6.16 grams of reagent grade anhydrous sodium acetate in water and diluting to 500 ml. (*4*).

***p*-Nitrophenol Indicator,** 0.1% aqueous solution.

Tris(ethylenediamine) Cobalt(III) Triphosphate, [$Co(en)_3H_2P_3O_{10}.2H_2O$]. This was prepared by dissolving 1.13 grams of sodium triphosphate hexahydrate in 125 ml. of water in a 250-ml. beaker. The solution was neutralized to *p*-nitrophenol with 0.5*N* hydrochloric acid, and 25 ml. of stock buffer solution was added. Then 25 ml. of tris(ethylenediamine) cobalt(III) chloride solution was added in 2-ml. portions with good stirring. The precipitate was filtered on a medium porosity fritted-glass funnel, washed with cold water, and air-dried.

Acids and Bases. Approximately 0.5*N* hydrochloric acid and 0.5*N* sodium hydroxide, without standardization; 0.10*N* carbonate-free sodium hydroxide, accurately standardized; and reagent grade nitric acid were used.

Sodium Sulfate. A stock solution was prepared by dissolving 1.050 grams of reagent grade anhydrous sodium sulfate in water and diluting to 100 ml.

APPARATUS

A Bausch & Lomb monochromatic colorimeter equipped with 13-mm. square cells and employing an interference filter having a maximum transmission at 455 mμ.

Burrell wrist action shaking machine, Model BB.

Eastman Kodak photo timer.

Beckman Model H pH meter, line operated.

A vacuum filtration apparatus, in which the funnel has a bent stem so that the filtrate can be collected in the bottom of the 1-liter suction flask or in the 25 × 170 mm. test tube contained therein.

Medium and coarse porosity fritted glass crucibles, 30-ml. capacity.

DEVELOPMENT OF THE METHOD

Measurement of Excess Reagent. Because the reagent is colored, it was decided to precipitate the triphosphate with an

Table I. Composition of Calibration Curve Solutions

$Na_5P_3O_{10}$/100 Ml., Gram	$Na_5P_3O_{10}.6H_2O$ Soln., Ml. (2.2637 G./100 Ml.)	$Na_4P_2O_7$ Soln., Ml. (0.7000 G./100 Ml.)	Na_2SO_4 Soln., Ml.
0.3500	50	0	10
0.3150	45	5	10
0.2800	40	10	10
0.2450	35	15	10
0.2100	30	20	10
0.1750	25	25	10

Table II. Effect of High Orthophosphate Content on Triphosphate Determination

Taken, %			$Na_5P_3O_{10}$, %	Diff.,
Na_2HPO_4	$Na_4P_2O_7$	$Na_5P_3O_{10}$	Found	%
5	0	45.0	44.6	−0.4
5	10	35.0	35.6	+0.6
5	20	25.0	25.7	+0.7
10	0	40.0	39.6	−0.4
10	5	35.0	34.9	−0.1
10	15	25.0	25.9	+0.9
25	0	25.0	25.3	+0.3
20	0	30.0	29.6	−0.4
15	0	35.0	34.1	−0.9

Table III. Effect of High Trimetaphosphate Content on Triphosphate Determination

Taken, %				$Na_5P_3O_{10}$, %	Diff.,
Na_2HPO_4	$Na_4P_2O_7$	$Na_3P_3O_9$	$Na_5P_3O_{10}$	Found	%
2	16	2	80.0	79.8	−0.2
2	12	6	80.0	80.8	+0.8
6	16	6	72.0	72.8	+0.8
6	20	2	72.0	72.6	+0.6
0	0	50	50.0	50.0	0.0
0	0	30	70.0	70.0	0.0
0	20	20	60.0	60.0	0.0

excess of reagent, then measure the excess of reagent colorimetrically. A series of absorption curves run with a Cary recording spectrophotometer (Model 11 MS) on solutions of different reagent concentration at pH 3.6 showed an absorption maximum at 465 mμ. The Bausch & Lomb monochromatic colorimeter has a filter with a maximum transmission at 455 mμ; this filter was used in carrying out the determinations.

Control of pH. Previous workers (*8*) have shown that the precipitation of triphosphate with tris(ethylenediamine) cobalt(III) ion is nearly quantitative between pH 3 and pH 4, and that the completeness of precipitation falls rapidly outside this range. A sodium acetate–acetic acid buffer (*4*) giving a pH of 3.6 was used to ensure maximum completeness of precipitation.

Calibration Curve. Because pyrophosphate has been shown to coprecipitate with triphosphate, a calibration curve was prepared using mixtures of these two materials. The amount of the material used is shown in Table I. A constant amount of reagent was always employed in carrying out the precipitation and the total amount of phosphates in the precipitation flask was equivalent to 0.20 ± 0.02 gram of phosphorus pentoxide. With these conditions being carefully controlled, the amount of reagent precipitated was proportional to the amount of triphosphate present.

Precipitation Procedure. A rigid precipitation procedure was required to ensure reproducible results. After placing the sample in the reaction flask, neutralizing and buffering it, the method of adding the reagent must be carefully controlled. The warming and seeding of the solution, slow addition of reagent, and regular shaking were used to form the precipitate slowly so that its composition was constant and contamination was minimized.

Effect of Anionic Detergent. The major portion of the anionic detergent was removed by dissolving it in 95% alcohol and filtering. The alcohol-insoluble phosphates were used in carrying out the triphosphate determination. Less than 0.3% of the anionic detergent was not removed by the alcohol separation. This was enough to retard precipitation at room temperature but not at 40° C.

Effect of Sodium Silicate. Sodium silicate was partly removed by adjusting the pH of the phosphate solution to the phenolphthalein end point and removing the precipitate by filtration. Simulated phosphate built detergent mixtures with and without soluble silicate were analyzed and no difference in results was obtained.

Effect of Sodium Sulfate. Although good results were easily obtained on commercial triphosphate samples, low results were found on built detergent samples. The deviation from the true value increased as the amount of triphosphate present decreased. It was shown that the presence of sodium sulfate caused this effect. A series of calibration curves was prepared, varying the sodium sulfate concentration (Figure 1). When no sodium sulfate was present, more than the theoretical amount of reagent was used, owing to the coprecipitation of pyrophosphate. As the concentration of sodium sulfate was increased, the amount of reagent used, and hence the completeness of precipitation, decreased. When less than 50% of the phosphate present was triphosphate, this effect was particularly pronounced and a point was reached where no precipitate was obtained. The effect was definitely due to the sodium sulfate, because the precipitation could be obtained in the presence of pyrophosphate alone down to a point where only 20% of the phosphate was triphosphate. It seems likely that this effect is due to the increased concentration of sodium ions which tend to form a soluble complex with triphosphate ions (*13*).

The concentration of sodium sulfate used in preparing curve 4 of Figure 1 was found satisfactory for running a variety of phosphate built detergents and consequently was used in making calibration curves (Table I). When commercial triphosphate samples were analyzed, this same amount of sodium sulfate solution was placed in the precipitation flask before precipitation was started, so that the same calibration curve could be used for all samples. Although the effect is not large, approximately the same amount of sodium sulfate should be used in setting up the calibration curve as will be present in the samples.

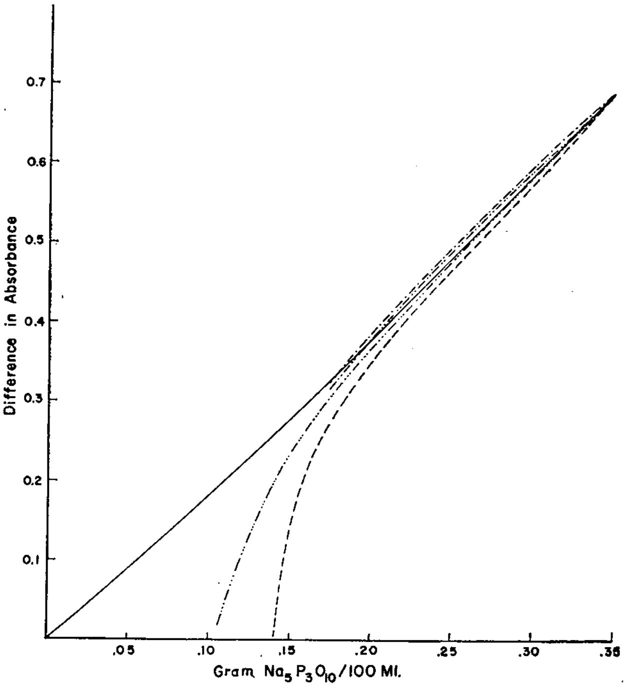

Figure 1. Effect of sodium sulfate on triphosphate calibration curves

1	———	Theoretical
2	—·—·—·	Weight ratio, sodium sulfate to phosphate, equals 0 to 50
3	—··—··—	Weight ratio, sodium sulfate to phosphate, equals 5 to 50
4	—···—···	Weight ratio, sodium sulfate to phosphate, equals 15 to 50
5	– – – – –	Weight ratio, sodium sulfate to phosphate, equals 30 to 50

Effect of Other Phosphates. Because the calibration curve was prepared using mixtures of tri- and pyrophosphate, the effect of large amounts of ortho- and trimetaphosphates was checked. The composition of the mixtures and the results obtained are presented in Tables II and III. Although the ortho- and trimetaphosphate contents vary over a wide range, even completely replacing the pyrophosphate, the average deviation for all the samples in Tables II and III is 0.44% absolute.

The presence of phosphate glass interferes with the triphosphate precipitation. (In this case, phosphate glass means any of the more condensed phosphates having average chain lengths equal to tetraphosphate or greater.) The degree of interference increases as the average chain length of the phosphate glass increases. Below the 1% level (for Calgon) little or no interference is observed, but at the 1% level a serious decrease in the completeness of precipitation occurs, and at higher levels no precipitation occurs. Quadrafos causes no interference at the 1% level but interferes at the 2.5% level. Tetraphosphate shows no interference at the 5% level but completely prevents precipitation when present at the 10% level. The degree of interference is apparent in one of the following ways:

1. If the determined sodium triphosphate content of a commercial triphosphate is less than 85%, which is abnormally low, it may indicate the presence of a small amount of phosphate glass. This interference is easily eliminated by the same treatment used when the triphosphate content is less than 50% (see Procedure).
2. If no precipitation occurs after 4 ml. of reagent have been added, but does occur when the remainder of the reagent is added, this indicates a greater degree of contamination than was present in (1). However, the interference usually may be eliminated by the method described in the Procedure.
3. If no precipitation occurs at any time during the addition of reagent, this indicates that the phosphate glass content is so large that the addition of triphosphate probably will not eliminate the interference. At the present time there is no variation of this method capable of analyzing such mixtures.

Thus, the method gives warning when this type of interference takes place and the analyst is not misled. Although the effect is serious, the phosphate glass content of commercial triphosphate is generally below the interfering level. A survey (Table X) of 22 samples from six triphosphate manufacturers showed only 3 samples containing interfering amounts of phosphate glass. The interference was eliminated in all cases by the addition of pure sodium triphosphate hexahydrate as described below.

COMPOSITION OF PRECIPITATE

Previous workers (*8*) have shown that pyrophosphate contaminates the triphosphate precipitate, yet gives a calibration curve in reasonably good agreement with the theoretical curve; therefore, it was decided to investigate the composition of the precipitate. The need for such information was emphasized by the good results obtained on samples containing large amounts of ortho- and trimetaphosphate (Tables II and III). Two sets of experiments were run. In the first the pyrophosphate was tagged with phosphorus-32 and its contamination of the precipitate was measured. In the second series the triphosphate was tagged and the amount of triphosphate in the filtrate measured by counting. The results obtained are presented in Table IV.

Assuming that no radiochemical exchange took place (experiments with the sodium phosphates in solution indicate that none does) the data show that: (1) the presence of sodium sulfate has little effect on the degree of pyrophosphate contamination of the precipitate, though other data (Figure 1) show that it does affect the amount of reagent consumed; (2) ortho- and trimetaphosphate have little effect on the completeness of triphosphate precipitation; and (3) the degree of pyrophosphate contamination of the precipitate and the degree of solubilization of triphosphate is significant; both of these increase as the ratio of pyrophosphate to triphosphate increases. The replacement of triphosphate by pyrophosphate in the precipitate is such that reagent usage is proportional to the concentration of triphosphate present. The mechanism of this replacement is not known but replicate determinations, both colorimetric and radiochemical, carried out using the method described below, indicate that contamination is constant and is reproducible at any particular triphosphate level. Reagent usage is proportional to the triphosphate concentration.

PROCEDURE

Preparation of Calibration Curve. Place the required amounts of triphosphate, pyrophosphate, and sodium sulfate solutions (Table I) in 100-ml. volumetric flasks. Prepare two 100-ml. volumetric flasks for blanks by placing 50 ml. of water and 10 ml. of sodium sulfate solution in them.

Add 4 drops of *p*-nitrophenol indicator to each flask and add 0.5*N* hydrochloric acid until the indicator is just decolorized. Add 10 ml. of stock buffer solution to each flask. Make the buffer blank up to the mark with distilled water, mix well, and use this solution to set the colorimeter to zero absorbance at 455 mμ. Add 10.0 ml. of reagent solution to the reagent blank flask, make up to volume with distilled water, mix well, and read absorbance with the samples.

Heat the contents of the remaining flasks to 40° C. by placing them in a hot water bath, and then attach them to the shaking machine. Add a few seed crystals (enough to cover a pin head) of tris(ethylenediamine) cobalt(III) triphosphate. Add 10.0 ml. of reagent from a 25-ml. buret in approximately 1-ml. portions, shaking for 60 seconds (use a timer) between additions, except after the fourth addition when the solution is shaken 15 minutes. After exactly 10.0 ml. of reagent have been added, reheat the solution to 40° C. and shake 15 minutes.

Cool the solution to room temperature and make up to volume with distilled water. Mix well and filter through a dry, medium porosity fritted-glass crucible, rejecting the first 30 ml. and collecting the second 30 ml. in a clean, dry test tube. Do not suck precipitate on the crucible dry at any time.

Pour the filtrate into a colorimeter cell and measure the absorbance after setting the instrument to zero absorbance with the buffer blank. Measure the absorbance of the reagent blank; this should be kept as constant as possible.

Prepare the calibration curve by plotting the difference between the absorbance of the reagent blank and that of the sample against the weight of sodium triphosphate ($Na_5P_3O_{10}$) in the 100 ml. of solution from which it was precipitated. Draw a line through the points. This line will have a slight curvature.

Preparation of Sample. COMMERCIAL TRIPHOSPHATE. Dissolve 3.500 ± 0.001 grams of sample in distilled water and dilute to volume in a 500-ml. volumetric flask. Mix well. This is the triphosphate sample solution.

DETERGENT SAMPLE. Using a stirring rod, completely disperse 3.500 ± 0.001 grams of triphosphate built detergent in 25 ml. of water at room temperature in a 600-ml. beaker. From a dropping funnel add 25 ml. of ethyl alcohol dropwise with good stirring, then add 375 ml. of ethyl alcohol with good stirring. Filter through a coarse fritted-glass crucible, refiltering the first portion if it comes through cloudy. Wash the precipitate

Table IV. Radiochemical Results

Sample	Sample Composition					Pyrophosphate in Precipitate as $Co(en)_3HP_2O_7$	Total $Na_5P_3O_{10}$ in Filtrate, %
	$Na_5P_3O_{10}$	$Na_4P_2O_7$	Na_2HPO_4	$Na_3P_3O_9$	Na_2SO_4		
1	45	5	..	..	..	2.15	..
2	45	5	..	..	15	2.36	..
3	50	50	..	..	..	10.8	..
4	50	50	..	..	15	12.1	..
5	45	5	..	..	15	..	2.3
6	40	10	..	..	15	..	4.2
7	25	25	..	..	15	..	12.5
8	45	..	5	..	15	..	0.8
9	25	..	25	..	15	..	1.3
10	25	..	..	25	15	..	1.3

with 100 ml. of ethyl alcohol, mixing the alcohol and precipitate well. Dissolve the alcohol-insoluble residue in 100 to 150 ml. of water by placing the filter funnel on a clean dry suction flask and sucking the water through the crucible. Add a Whatman filter accelerator to the solution and disperse it with a stirring rod. Transfer the suspension to a 200-ml. volumetric flask. Add 1 drop of phenolphthalein and then 0.5*N* hydrochloric acid with shaking until the pink color is just discharged. Dilute to the mark with water and mix well. Filter through a dry 12.5-cm. Whatman No. 31 filter paper, discarding the first 25 ml. The main portion of this filtrate is the detergent sample solution.

Determination of Total Phosphorus Pentoxide. Accurately transfer 25.0 ml. of the triphosphate sample solution or 20.0 ml. of the detergent sample solution to a 400-ml. beaker. Carry out the acid hydrolysis and two end point titrations according to the method reported by Andrews (*1*), using 0.1*N* sodium hydroxide as the titrant. Because the phosphorus pentoxide content of commercial triphosphate samples is nearly constant, it is generally not necessary to run this determination before the triphosphate determination; however, it must be run first on detergent samples.

Determination of Triphosphate. The aliquot for the triphosphate determination must contain 0.20 ± 0.02 gram of phosphorus pentoxide. Calculate the volume of detergent sample solution required using the following formula:

$$\text{Ml. of sample} = \frac{11.43}{\%\ P_2O_5} \times 100$$

Generally, 50 ml. of the triphosphate sample solution can be used, but if the total phosphorus pentoxide content of the sample is less than 52% a proportionally larger sample must be weighed. The detergent sample solution should be placed in a clean, dry 50-ml. buret and the calculated volume (to the nearest milliliter) transferred to a 100-ml. volumetric flask.

Bring the solution volume to about 50 ml. by adding water. If the samples are commercial triphosphate, add 10 ml. of sodium sulfate solution. Prepare two 100-ml. volumetric flasks for blanks by placing 50 ml. of water and 10 ml. of sodium sulfate solution in them. Carry out the neutralization, precipitation, and preparation of blanks as described for preparation of the calibration curve. After the filtration measure the absorbance of the sample solution. Subtract the sample absorbance from the reagent blank absorbance and locate the triphosphate ($Na_5P_3O_{10}$) content of the aliquot on the calibration curve. Calculate the triphosphate content as follows:

$$\%\ Na_5P_3O_{10} = \frac{\text{grams of } Na_5P_3O_{10} \text{ in aliquot} \times 2000}{3.5 \times \text{ml. in aliquot}}$$

If no precipitate forms, or the difference in absorbance falls below the end of the calibration curve, or if the triphosphate content is known to be less than 50% of the phosphate present in the sample, take one half of the calculated aliquot and add 0.2237 gram of pure sodium triphosphate hexahydrate (equivalent to 0.173 gram of the anhydrous sodium triphosphate) and 5.0 ml. of sodium sulfate solution. Carry out the procedure as described above, but after determining the amount of triphosphate in the aliquot from the calibration curve, calculate the per cent triphosphate as follows:

$$\%\ Na_5P_3O_{10} = \frac{(\text{grams of } Na_5P_3O_{10} \text{ in aliquot} - 0.173)\ 2000}{3.5 \times \text{ml. in aliquot}}$$

This procedure is also useful if the calculated aliquot is so large that the final volume of the precipitating solution will be greater than 100 ml.

Determination of Orthophosphate. The method of Martin and Doty (*9*) is recommended. With some slight modifications it has given an average deviation of 0.05% absolute from the true value at the 1% level when applied to simulated mixtures of commercial triphosphate composition.

Determination of Trimetaphosphate in Commercial Triphosphate. Trimetaphosphate was determined by the method of Jones (*5*) with some slight modifications (barium precipitation was carried out at pH 10), and the unprecipitated phosphate was determined by the Martin and Doty (*9*) colorimetric orthophosphate method after hydrolysis. When used on simulated samples of commercial triphosphate composition containing known amounts (1 to 3%) of trimetaphosphate, the results showed an average deviation of 0.03% from the true value.

Table V. Accuracy of Triphosphate Determination on Simulated Commercial Triphosphate

Taken, %				$Na_5P_3O_{10}$, % Found	Diff., %
Na_2HPO_4	$Na_4P_2O_7$	$Na_3P_3O_9$	$Na_5P_3O_{10}$		
20	20	0	60.0	60.8	+0.8
8	24	0	68.0	68.5	+0.5
1	24	0	75.0	76.2	+1.2
1	13.6	3	83.0	83.7	+0.7
0	9	3	88.0	88.9	+0.9
1	0	3	96.0	95.1	−0.9
0	0	3	97.0	96.6	−0.4

Table VI. Accuracy of Triphosphate Determination on Simulated Built Detergents

% $Na_5P_3O_{10}$		Diff., %
Taken	Found	
49.0	48.3	−0.7
44.0	43.1	−0.9
39.0	39.1	+0.1
34.0	34.1	+0.1
29.0	29.1	+0.1
24.0	23.9	−0.1
19.0	18.1, 17.8	−0.9, −1.2
14.0	13.1, 13.9	−0.9, −0.1
9.0	7.7, 7.7	−1.3, −1.3
4.0	3.6, 5.0	−0.4, +1.0
0.0	0.3, 0.2	+0.3, +0.2

Calculation of Pyrophosphate Content. After determining the total phosphorus pentoxide, ortho-, tri-, and trimetaphosphate contents of the sample, the pyrophosphate content can be estimated by difference using the following equation:

$$\%\ Na_4P_2O_7 = (T - O - T_2 - T_3) \times 1.87$$

where $T = \%\ P_2O_5$
$O = \%\ Na_2HPO_4 \times 0.500$
$T_2 = \%\ Na_3P_3O_9 \times 0.696$
$T_3 = \%\ Na_5P_3O_{10} \times 0.579$

When this calculation was applied to a series of simulated detergent mixtures containing known amounts of the phosphates, which had been analyzed for ortho- and triphosphate and total phosphorus pentoxide by the above methods, the pyrophosphate results showed an average deviation of 0.9% absolute from the mean. The pyrophosphate content ranged from 0 to 49%. Being a difference figure, the accuracy is considerably less than can be obtained for a direct determination. There is still a great need for a direct method of determining pyrophosphate.

DISCUSSION

Accuracy and Reproducibility. Simulated commercial triphosphate samples were prepared by weighing the calculated amounts of the pure phosphates, dissolving the mixture in water, and analyzing the resulting solution. The results obtained are presented in Table V. The data show an average deviation of 0.8% absolute.

The reproducibility of the method was estimated by making replicate determinations on a single commercial triphosphate sample. Seven determinations gave an average value of 89.8% sodium triphosphate and an average deviation of 0.53% absolute from the mean, with a standard deviation of 0.59% absolute. The spread between the highest and lowest values was 1.5% absolute.

The method was tested for accuracy on detergents by analyzing simulated mixtures in which the components were weighed into the mixture. Fifty per cent of the sample was a spray-dried unbuilt powder containing sodium alkylbenzene sulfonate, sodium sulfate, and sodium silicate. The remaining half of the sample was composed of 1% disodium orthophosphate, the amount of sodium triphosphate shown in Table VI, and sufficient sodium pyrophosphate to provide the balance of the sample. The results (Table VI) show an average deviation of 0.61% absolute. The values below 24% in Table VI show a larger average deviation (0.76%) than those containing 24% or more sodium triphosphate (0.3% average deviation). This is due, at least in part, to the need for adding pure sodium triphosphate hexahydrate to make the triphosphate content at least 50% of the phosphates present, and then making a correction for this addition. When the standard deviation was calculated for all the known compositions analyzed (Tables II, III, V, and VI) a value of 0.8% absolute was obtained. This is considered to be a good measure of the accuracy of the method.

The reproducibility of the method when applied to triphosphate built detergents was ascertained by running eight determinations on a single sample of a commercial detergent. Four individual portions were weighed, and after the alcohol separation, the triphosphate determination was run on duplicate aliquots. A mean value of 36.1% sodium triphosphate was found and the results showed an average deviation of 0.31% absolute from the mean, with a standard deviation of 0.38% absolute. The spread between the highest and lowest values was 1.1% absolute.

The results obtained through replicate analyses on a single commercial triphosphate sample are presented in Table VII. Four samples were weighed and dissolved and duplicate determinations were run on each solution. These data show the reproducibility which may be expected from each of these determinations.

Comparison with Isotope Dilution Method (*12*). A large number of commercial triphosphate and built detergent samples were run by both methods. (The isotope dilution analyses were run by the originators of that method.) The results are presented in Tables VIII and IX. The results obtained by the two methods are in reasonably good agreement. The isotope dilution results tend to be higher than the colorimetric results. The standard deviation reported for the isotope dilution method was 1.5% absolute with a bias not exceeding +2% absolute.

Table VII. Replicate Analyses on Commercial Triphosphate

Determination No.	Na_2HPO_4, %	$Na_5P_3O_{10}$, %	$Na_3P_3O_9$, %	P_2O_5, %	$Na_4P_2O_7$, %
1*a*	0.56	90.0	1.07	56.98	6.81
b	0.56	[b]	1.06	57.56	7.86
2*a*	0.56	89.4	1.06	57.56	8.68
b	0.60	89.1	1.06	57.60	8.95
3*a*	0.56	89.7	1.08	57.43	8.14
b	0.56	89.1	1.07	57.23	8.37
4*a*	0.64	90.6	1.01	58.41[a]	8.91
b	0.64	90.6	1.05	58.54[a]	
Mean	0.59	89.8	1.06	57.39	8.14
Av. dev. from mean	0.03	0.53	0.01	0.19	0.53
Range	0.08	1.5	0.07	0.62	2.14

[a] Obtained by different method; not included in calculations.
[b] Lost by operational error.

Table VIII. Triphosphate Content of Commercial Triphosphate by Colorimetric and Isotope Dilution Methods

	Sample No.				
	1	2	3	4	5
Colorimetric, % $Na_5P_3O_{10}$	90.6	91.2	90.0	91.2	88.1
Isotope dilution, % $Na_5P_3O_{10}$	91.0 91.5 88.5	93.4 91.1 90.3	92.7 87.0 86.0 88.0 86.2	91.8 90.6 92.0	89.7 88.5 89.4

Table IX. Triphosphate Content of Commercial Built Detergents by Colorimetric and Isotope Dilution Methods

	Sample No.					
	1	2	3	4	5	6
Colorimetric, % $Na_5P_3O_{10}$	37.4 37.6 37.1 37.6	36.4 36.7 38.1 38.0	39.9 40.6 40.3	39.9 41.0 40.4	19.2 19.4 19.6 19.1 19.8	19.4 19.7 19.6 19.8
Isotope dilution, % $Na_5P_3O_{10}$	38.1 41.4	32.6 33.0 36.2	42.7 42.3 41.2	39.9 42.4	20.2 20.0	19.6 17.0

Table X. Composition of Commercial Triphosphate Samples

Supplier	Na_2HPO_4, %	$Na_3P_3O_9$, %	$Na_5P_3O_{10}$, %	P_2O_5, %	$Na_4P_2O_7$, %	Sum of Phosphates
I	0.61	0.66	90.0	57.02	7.8	99.07
	0.04	0.57	90.6	56.58	7.4	98.60
	0.88	1.88	86.0	56.57	9.4	98.16
	1.36	1.55	86.0	57.39	10.9	99.81
	0.47	0.61	92.9	56.98	4.7	98.70
	0.40	0.63	95.4[a]	57.83	3.7	100.08
II	0.29	1.32	86.6	57.14	11.1	99.31
	0.22	2.15	89.4	56.93	6.7	98.47
	0.26	1.25	86.6	56.77	10.5	98.61
	0.64	1.84	84.6	57.18	12.4	99.48
	0.26	0.71	89.2[a]	57.63	10.0	100.17
	0.26	0.80	88.6[a]	57.43	10.2	99.86
III	0.15	0.06	92.8	56.00	4.1	97.10
	0.20	0.05	90.0	55.92	5.1	95.25
	0.41	0.07	89.7	55.74	6.7	96.83
	0.23	0.42	93.1	55.67	2.5	96.20
IV	0.26	3.50	90.0	57.08	4.5	98.26
	0.20	1.88	92.3	57.43	4.8	99.18
V	0.26	0	88.3	57.10	10.9	99.46
	0.23	0.14	88.3	57.06	10.7	99.37
VI	0.20	1.57	85.7	56.98	5.29	97.17
	0.08	1.59	88.0	57.26	5.1	99.27
High	1.36	3.50	95.4	57.63	12.4	100.17
Low	0.04	0.0	84.6	55.67	2.5	95.25
Av.	0.36	1.06	89.3	56.90	7.5	98.56

[a] Obtained by using one half of aliquot and adding pure $Na_5P_3O_{10} \cdot 6H_2O$.

Application to Commercial Triphosphate. Twenty-two samples of commercial triphosphate prepared by six different manufacturers in nine factories were analyzed by the methods outlined above. The results are presented in Table X. Only three samples contained interfering amounts of phosphate glass, which was eliminated in each case by the procedure given previously. The high, low, and average values are also listed in Table X. Although there is considerable variation in the results, there is no indication that these are the fault of the methods used. Some of the variations can be explained by the method of manufacturing the product. For example, the low "Sum of Phosphates" values found for one manufacturer are caused by the use of wet process phosphoric acid which normally contains some sulfate.

ACKNOWLEDGMENT

The author wishes to express his appreciation to F. L. Jackson and H. W. Lampe for their advice and assistance in carrying out the radiochemical experiments, and to O. T. Quimby, H. W. McCune, and H. V. Meek for their advice and encouragement during the development of the method. The assistance of Julianna Fey, who performed most of the analyses, is gratefully acknowledged.

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RECEIVED for review November 12, 1954. Accepted January 13, 1956. Division of Analytical Chemistry, 126th Meeting, ACS, New York, September 1954.

Deuterization of Steroids and Their Use in Isotope Dilution Analysis

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A workable and reliable isotope dilution assay for the steroids, compound S and compound F, in fermentation liquors has been developed. Data on the catalytic introduction of deuterium into several new steroids, some of them cortical hormones, are presented.

IN STUDIES of the microbial oxidation of compound S (17-hydroxy-11-desoxycorticosterone) to compound F (17-hydroxycorticosterone) in the Merck laboratories, it became necessary to know accurately the amounts of the above steroids in the fermentation liquors. Methods dependent upon quantitative recovery of the steroids from the fermentation broths presented almost insurmountable difficulties because of the complex nature of the medium. An isotope dilution technique, on the other hand, requires only that a representative pure specimen of the substance for which assay is sought be obtained. The necessity for quantitative recovery is avoided.

Previous experience led to the selection of deuterium as the tracing isotope. The deuterium was introduced into the steroid molecule by a technique similar to that used by Fukushima and Gallagher (*6*) in which platinum catalyst and 70% deuterioacetic acid was used. Known amounts of deuterio compound S (17-hydroxy-11-desoxycorticosterone) and deuterio compound F (17-hydroxycorticosterone) were added directly to the whole fermentation liquor, intimately mixed, then extracted, separated, and purified. From the dilution of the deuterium in the isolated steroids, the original steroid content of the broth can be calculated.

REAGENTS

Compound F (hydrocortisone or 17-hydroxycorticosterone); compound S (17-hydroxy-11-desoxycorticosterone); compound E (cortisone or 17-hydroxy-11-dehydrocorticosterone); Δ^1-dehydro-17-hydroxycorticosterone; and 4,5-dihydro-17-hydroxy-11-dehydrocorticosterone were prepared. These compounds were all converted to the 21 acetates for deuterization to improve their stability. The deuterized F and S acetates were subsequently hydrolyzed to the corresponding alcohols for use in analysis. The catalyst, consisting of 13% platinum on acid-washed charcoal, was prepared as described by Trenner (*7*). Deuterioacetic acid (70% by weight) was prepared by mixing the proper proportions of Merck reagent grade acetic anhydride and 99.5% deuterium oxide obtained from the Stuart Oxygen Co. (by license from the Atomic Energy Commission).

PREPARATION OF TRACERS

The steroids listed in Table I were all deuterized by the platinum catalyst-deuterioacetic acid method previously described (*1–3*, *5*, *6*). The reaction mixture consisting of steroid, catalyst, and deuterioacetic acid, was placed in a large borosilicate glass ampoule equipped with a narrow neck and containing a magnetic stirring bar. The ampoule was immersed in a dry ice–acetone bath, evacuated, and sealed, prior to being placed in the heating bath. The reaction mixture was constantly stirred by the magnetic stirrer and heated by the vapor of a refluxing liquid of the appropriate boiling point. After the proper time, the contents of the ampoule were filtered through a medium porosity sintered-glass funnel and the residue was washed with four 25-ml. portions of acetone, each wash being allowed to stand with the residue about 30 minutes before filtering. This procedure aids in the desorption of deuterized steroid from the charcoal.

The combined filtrates were vacuum-distilled to near dryness, then the residue was dissolved in 100 ml. of methanol and permitted to stand overnight to remove labile deuterium. The methanol was finally removed by vacuum distillation and the product was recrystallized from ethyl acetate.

The infrared absorption of the product was examined in the O-D region at 3.98 microns to assure the absence of detectable amounts of labile deuterium of this kind. The absence of other kinds of labile deuterium is demonstrated by the data in Table II.

The F and S acetate tracers were hydrolyzed to the corresponding alcohols for use in the analysis of broth. The hydrolysis was carried out as follows: The acetate was dissolved in purified methanol by warming to 50° C. and then cooling to 30° C. Sodium methoxide, dissolved in purified methanol, was added with stirring under nitrogen. After 9 minutes, dilute acetic acid was added until the solution was just acid to litmus. The steroid alcohol was isolated by removing most of the methanol by vacuum distillation, adding water, and letting the crystallization take place in the refrigerator. The product was filtered, dried, and recrystallized from ethyl acetate.

The atom per cent deuterium in the final product is determined by combustion of the steroid and assay of the resulting water by infrared spectrophotometry, essentially along the lines described by Trenner, Arison, and Walker (*8*). Since the appearance of this article (*8*), a modified procedure of greater precision and convenience has been developed (*9*).

The same batch of catalyst was used in all experiments of Table I except experiments 3 and 4. The 70% deuterioacetic acid was prepared by stirring for 1 hour 80 ml. of acetic anhydride and 56 ml. of deuterium oxide (99.5%) at room temperature. Quoted yields are crude products before recrystallization from ethyl acetate.

COUNTERCURRENT SEPARATION OF COMPOUNDS F AND S

In order to separate compounds F and S alcohols, a countercurrent system using butyl alcohol–iso-octane as the upper phase, and water–butyl alcohol as the lower phase was developed. The system is prepared by equilibrating equal volumes of 20% butyl alcohol in iso-octane (by volume) and water. At equilibrium about one third of the butyl alcohol is in the lower, aqueous phase. In this system compound S free alcohol has a distribution ratio of about 7.3 (defined as concentration in upper phase over concentration in lower phase), while compound F free alcohol has a distribution ratio of about 0.57. Thus, in a five-transfer (six-tube, numbered 0, 1, 2, 3, 4, 5) system, moving the lower (aqueous) phase, the above steroids distribute in such a way that tube zero, at the end of the distribution, contains approximately 53% of the compound S alcohol and less than 1% of the compound F alcohol in the total system. Tubes 3 and 4, on the other hand, contain a total of about 63% of the compound F alcohol, and less than 1% of the compound S alcohol (*4*).

PROCEDURE

An amount of whole fermentation broth estimated to contain 100 mg. of compound F alcohol is chosen as the sample and is placed in a high speed (Waring) blender. One hundred milligrams each of deuterio compound F and deuterio compound S in methanol solution are added to the broth sample and the whole is agitated in the blender for about 20 minutes. The mixture is then transferred to a round-bottomed flask and reduced by vacuum distillation to about one fifth of its original volume. Thus with an original sample of 250 ml., 50 ml. or less will remain at this point.

The concentrate is now placed in the blender again and blended about 10 minutes with 500 ml. of ethylene dichloride. The slurry is separated in a separatory funnel and the ethylene di-

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Table I. Preparation of Deuterized Steroids

Expt. No.	Steroid Added (Acetates)	Weight of Steroid Added, Grams	Weight of Catalyst, Gram	% D in Deuterio-acetic Acid	Volume D Acetic Acid Added, Ml.	Temp., ° C.	Time, Hours	% D Found	Crude Yield, %
1	Compound F	2.0	0.4	70	30	82	67	8.7	85
2	Compound F	2.0	0.8	70	30	82	70	8.2	59
3	Compound S	1.0	0.2	60	23	76	72	6.1	43
4	Compound S	2.0	0.37	70	25	64	168	7.5	58
5	Δ_1-Dehydro F	2.0	0.4	70	30	82	69	1.7	59
6	Compound E	2.0	0.4	70	30	82	69	13.3	87
7	4,5-Dihydro E	2.0	0.4	70	30	82	70	9.0	90

chloride phase is filtered on a Büchner funnel. The clear, usually yellow ethylene dichloride filtrate is washed with two 250-ml. portions of saturated sodium bicarbonate solution and finally evaporated to dryness on the steam bath under a stream of nitrogen.

The dried steroid residue is now washed with two 100-ml. portions of petroleum ether, dried, and then dissolved in the number zero tube containing the 20% butyl alcohol–iso-octane and water system, being warmed if necessary to achieve solution of the solids. A five-transfer system using 100-ml. phases in 300-ml. separatory funnels has been found satisfactory for the routine separation of the F and S alcohols. The lower, aqueous phase is the moving phase, and five transfers, involving six separatory funnels, are carried out.

Table II. Lability of Deuterium during Fermentation and Isolation

Expt. No.	S Alcohol Tracer Added, % D	S Alcohol Recovered, % D	F Alcohol Recovered, % D
1	9.2	...	8.6
2	9.1	9.0	8.9
3	9.1	...	8.8

Compound S alcohol, free from compound F alcohol, is recovered from the number zero separatory funnel, while compound F alcohol, substantially free from compound S alcohol, is recovered from combined tubes 3 and 4. The solvents are removed by vacuum distillation or steam bath evaporation under a stream of nitrogen.

The dried steroid residues are now dissolved in a small quantity of hot ethyl acetate, treated with small portions of activated charcoal and acid-washed alumina, centrifuged, and crystallized by evaporative concentration and refrigeration.

Table III. Effect of Blending Time on Assay

Expt. No.	Time in Blender, Minutes	F Alcohol Found, Mg.	S Alcohol Found, Mg.
1	5	34	117
2	20	34	117
3	30	41.5	117
4	5	27	114

After a second crystallization from ethyl acetate, and to effect further purification of these isolates, the steroid alcohols are converted to the acetates by the following procedure. Two tenths milliliter of dry pyridine and 0.04 ml. of acetic anhydride are added to the dry alcohol (approximately 100 mg.), and the container is placed in a heating bath maintained at 56° C. for 75 minutes. The mixture is then cooled, and 2.0 ml. of petroleum ether are added to precipitate the steroid acetate. The precipitated acetate is washed with petroleum ether, dried, and recrystallized twice from ethyl acetate. The final isolate is dried 2 hours at 100° C., under vacuum. The usual yield of crystalline acetate is about 15 mg., an amount more than sufficient for combustion and infrared determination of the deuterium present in the water formed. The melting point of the final product should be consistent with that of pure material.

The atom per cent deuterium, determined from the infrared absorption of the water of combustion at 3.98 microns (*8*), is corrected for the natural abundance where necessary, and then is used to calculate the amount of steroid originally present in the unknown sample.

$$W_s = \left(\frac{C_t}{C_f} - 1\right) \times W_t \times \frac{M_s}{M_t}$$

W_s = weight steroid originally present
W_t = weight tracer added
C_t = atom % deuterium in tracer
C_f = atom % deuterium found in isolate
M_s = molecular weight of sample steroid
M_t = molecular weight of tracer steroid

Since the tracer steroids are added to the sample as the alcohols, but isolated as the acetates, the value taken for C_t must be the atom per cent deuterium calculated for the acetates, not the alcohols.

EXPERIMENTAL RESULTS

The blending time for the sample and tracer was varied to determine whether or not mixing was complete. The results, all carried out on equal portions of the same sample, are shown in Table III. They show no significant difference, indicating that even 5 minutes are sufficient for satisfactory physical equilibration. In experiment 4, the mixture was heated to 100° C. after blending, and held at that temperature for 1 hour.

Table IV. Analysis of a Known Broth

Compound	Steroid Added, Mg.	Steroid Found, Mg.
F	87.5	90
S	68.2	64

Compound S alcohol containing deuterium was added to a fermentation broth at the beginning of the fermentation, to determine whether appreciable loss of isotope took place during the actual fermentation. The results of three such experiments, shown in Table II, indicate losses of only a few tenths atom per cent of deuterium, and are almost within experimental error. These results point up the fact that the tracers used contain no significant amounts of deuterium labile under any of the given experimental conditions.

A blank fermentation broth, fermented in the absence of added steroid, was used as a control for the over-all assay. To 250 ml. of such a blank broth known amounts of compound S and compound F alcohols were added, then the assay was carried out by the addition of tracer in the usual manner. The results of such a run, giving a good check on the reliability of the method, are shown in Table IV.

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RECEIVED for review October 8, 1955. Accepted January 25, 1956.

Liquid Scintillation Technique for Measuring Carbon-14–Dioxide Activity

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A method is described for the determination of carbon-14 in large amounts of carbon dioxide using a liquid scintillation counter. The carbon dioxide is dissolved in a toluene-methanol counting medium by diffusing it into a solution of a high molecular weight quaternary ammonium hydroxide. The efficiency of counting is independent of the amount of carbon dioxide, but does depend on the amount of amine. The efficiency ranges from 33% to greater than 67%.

Liquid scintillation counting is useful with β-emitting isotopes because of its high efficiency and relative freedom from loss of activity by self-absorption. Very large samples can be analyzed compared to those which can be used with Geiger counters, thus facilitating the practice of isolation with nonradioactive carriers and permitting the use of low levels of activity when a large sample is available. The latter is particularly useful for work with humans. Unfortunately, the composition of the solvent which may be used practically in scintillation systems is restricted to a small number of organic compounds. Toluene appears to be the preferred solvent, but there are many substances it cannot dissolve. The solubility problem is further aggravated by the need to operate the instrument below room temperature. In work with carbon-14, it is frequently necessary to measure the activity in carbon dioxide, a substance of distinctly limited solubility in toluene. The use of high pressure and very low temperatures (*9*) is not practical for routine work.

It has been found that carbon dioxide can be dissolved in toluene by combining it with a high molecular weight quaternary ammonium hydroxide. Samples as large as 5 mmoles. can be counted with good efficiency using a simple technique. Other toluene-insoluble acids can be dissolved similarly.

APPARATUS

A commercially available liquid scintillation counter incorporating a coincidence circuit and a 2-channel pulse height analyzer is used (Tri-Carb Counter, Model 314, Packard Instrument Co., LaGrange, Ill.). The samples and photomultiplier tubes are contained in a freezer maintained at about 5° C.

Samples are counted in $2^{1}/_{2}$-ounce cylindrical jars (Hazel-Atlas, No. 3296 from Crown Glass Corp., Chicago) fitted with black metal screw caps having "pulp and vinylite" liners. A roughly cut disk of tin foil, 0.001 inch thick and a little larger than the cap, is placed over the mouth of the jar before screwing on the cap. This prevents extraction from the cap of material which affects counting efficiency. Samples are readily labeled by writing or scratching on the cap. All-glass weighing bottles, used in early work, resulted in great breakage losses, difficulty in opening the bottles, and evaporation on storage. The use of silicone oil as a light pipe between the photomultiplier tubes and sample jar was abandoned when it was found that the oil was rapidly contaminated with light-absorbing materials, which resulted in lower and variable counting efficiencies. With carbon-14 and stronger β-emitters there is little loss in efficiency when the oil is omitted. The data reported here were obtained without oil.

A simple diffusion flask is used to transfer the carbon dioxide of each sample into the organic base. The flask is made (Delmar Scientific Laboratories, Chicago) from two 50-ml. Erlenmeyer flasks joined by a short tube of 16-mm. outside diameter at a point near the necks. To minimize the danger of spray carryover, the tube is made in the shape of an inverted V. The seals are made so that the two flasks sit firmly on any flat surface. One of the flasks has a side arm at right angles to the connecting tube, also near the neck. Three glass hooks, together with the side arm, permit the use of rubber bands to hold down the two rubber stoppers. For small scale work, a typical Warburg flask with enlarged center well can be used.

To speed the diffusion a shaker is used, such as a platform rotator (No. 16L14824, A. S. Aloe Co., St. Louis, Mo.).

MATERIALS

Silver oxide is prepared from equal volumes of 1*N* silver nitrate and 1*N* sodium hydroxide. The precipitate is washed twelve times by decantation with carbon dioxide–free water and then four times with carbon dioxide–free absolute methanol (redistilled ACS reagent grade), then dried in vacuo over calcium chloride. Mallinckrodt silver oxide may also be used.

The quaternary ammonium hydroxide is prepared from *p*-(diisobutylcresoxyethoxyethyl) dimethylbenzylammonium chloride (Hyamine crystals 10-X, Rohm & Haas, Philadelphia), which is first recrystallized from 4 volumes of toluene (J. T. Baker, ACS reagent grade). The chloride is converted to the free base by dissolving 252 grams (500 mmoles.) of the chloride in 750 ml. of absolute methanol, and adding 9 ml. of water and 63.3 grams of silver oxide. The suspension is swirled vigorously with the platform rotator for 30 minutes, then centrifuged, and the supernatant is transferred to a bottle.

Storage of the colorless solution usually results in yellowing and formation of a small amount of insoluble material, evidently derived from soluble silver complexes. Therefore, it is necessary to age the solution. This process is accelerated by exposing the bottle to a bright light for about 3 days. The supernatant is drawn off when further exposure produces no more deposition. This is most readily detected by tipping the bottle a little after each observation of the inner wall.

For carbon dioxide samples of about 1 mmole. or less, the methanolic solution can be used. However, methanol decreases the pulse sizes and for greater efficiency of counting at a given background rate, it is necessary to reduce the methanol content of the scintillation system. Evaporating the amine to dryness and dissolving in toluene produce a dark solution. A satisfactory solution (about 0.5*M*) can be obtained by titrating an aliquot with aqueous hydrochloric acid (using phenolphthalein), then evaporating off enough methanol to give approximately 1*M* base and adding an equal volume of toluene. The evaporation is done under vacuum in a water bath at room temperature using a "swirler" (*6*). At this point the solution may turn yellow within 1 day and will then slowly decolorize, depositing a gray precipitate. This is presumably due to an additional silver complex, but the color appears to have no effect on the counting efficiency.

The hydroxide solution is stored in a bottle fitted with a Teflon-glass stopcock and a soda-lime protection tube. The bottle is made by sealing an Ultramax double-port valve (Fischer and Porter Co., Hatboro, Pa.) at right angles to the outlet of an aspirator bottle (Corning Glass Works, Catalog No. 1220).

PROCEDURE

The required amount of hydroxide (at least 1.1 moles per mole of carbon dioxide) is placed in the flask without the side arm. The carbon dioxide, as carbonate in sodium hydroxide, an enzymic digest, or a slurry of barium carbonate, is then placed in the other flask. Excess sulfuric acid is next placed in the side arm with a curved pipet, the vessel is closed, the acid is tipped in, and the flask is shaken for 90 minutes.

After diffusion is completed, the amine–carbon dioxide solution is transferred to the counting jar by pipet, using two rinses of toluene to effect quantitative transfer. Toluene is added to bring the solution to 30 ml., then 5 ml. of a 2.1% solution of 2,5-diphenyloxazole in toluene (*4*) is added. The scintillator is obtained from Tracerlab, Boston. The jar is then capped and cooled in the counting freezer at least 45 minutes before counting. This delay is not imperative, but samples give a slightly lower count rate while warm. Because the samples are not phosphorescent it is not necessary to transfer the jars to the counting position in the dark.

A blank for background measurement is prepared by using the same amount of hydroxide and a solution of nonradioactive

Table I. Time Required for Complete Transfer of Carbon Dioxide in Diffusion Flasks[a]

Time, Minutes		Observed Activity, C.P.M.
30		14,363 14,903
	Av.	14,633
45		15,648
60		16,088 16,343
	Av.	16,216
75		16,418
90		16,608 16,448
	Av.	16,528

[a] Each flask contained 1 ml. of Hyamine solution and 1 ml. of sodium carbonate solution; activities have been corrected for background.

carbonate, with enough carbonate to neutralize most of the amine.

RESULTS AND DISCUSSION

Stability Experiments. Hydroxide solutions tested by titration showed a decrease in basicity of about 6% in 1 month, but the counting efficiency was unchanged. Radioactive and background samples gave the same activity readings over a period of 2 months or more, although fluctuations of a few per cent occurred, due to instrumental variation.

Diffusion Time. A series of similar diffusion flasks was shaken for varying times and the resultant amine solutions were counted (Table I). The observed activity levels off at about 75 minutes; however, 90 minutes is the recommended time. The samples should be withdrawn from the diffusion flask fairly soon after this time, as prolonged diffusion might cause sufficient diffusion of methanol and water to affect the counting efficiency of the Hyamine solution.

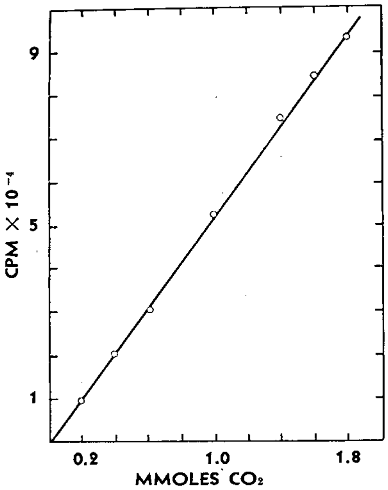

Figure 1. Relation between observed activity and amount of carbon-14–dioxide

Proportionality of Counts to Sample Size. The observed activity is independent of the amount of carbon dioxide in the sample. This is illustrated in Figure 1, which was derived by analyzing varying aliquots of a stock solution of radioactive sodium carbonate, using 2 mmoles. of hydroxide. This situation, which corresponds in Geiger counting to absence of a self-absorption correction and limit, means that exact control or knowledge of the actual amount of carbon dioxide present in the system is not necessary for an accurate count. Moreover, reagents need not be carbon dioxide–free if sufficient excess of hydroxide is used. Figure 1 shows that there is no need for a dead-time correction even at counting rates of 90,000 counts per minute.

Efficiency and Sensitivity of Counting. The efficiency of the counting technique decreases with increasing amounts of quaternary amine, as shown in Table II. A solution of known carbon-14 content, from the National Bureau of Standards, was used to convert activities to efficiencies. The two columns of efficiency data were obtained for pulse heights of 10 to 50 volts and 10 volts to infinity. At the lower levels of amine, the data on the 10 volt to infinity range are usually used; at the upper levels, 10 to 50 volts. The data in Table II also show that the counter efficiency increases as wider ranges of pulse heights are accepted. This effect arises from the distribution of pulse heights resulting from the spectrum of beta energies.

Because the background also increases with increased pulse height range, the maximum sensitivity is obtained by a compromise. In the system used here, the activity of a 0.9-mmole. sample of carbon dioxide is equal to background when the specific activity of the carbon is 1.3×10^{-5} μc. per milligram (background is 187 c.p.m. at 10 volts to infinity). Similarly, with 4.68 mmoles. of hydroxide and 4.2 mmoles. of carbon dioxide, the activity equals background when the specific activity is 2.8×10^{-6} μc. per milligram (background is 105 at 10 to 50 volts). Nine mmoles of carbon dioxide can be counted also, but only a small increase in sensitivity is obtained. For other instruments or even different models of the same instrument, the efficiencies and optimum range of pulse heights may vary from those presented here.

Table II. Counting Efficiency as Function of Amount of Quaternary Amine

Millimoles of Base	Efficiency[a] 10 to 50[b], %	10 to ∞ [b], %
0.468	40	67
0.936	41	60
1.40	44	58
1.87	41.5	53
2.81	38	47
3.74	36.5	42.5
4.68	33	38

[a] 100 × observed activity/absolute activity.
[b] Range of pulse heights counted, in volts.

Addition of 2.3 mg. of 2-(1-naphthyl)-5-phenyloxazole, a wave length shifter sometimes used in liquid scintillation counting (*1*), gave no increase in efficiency.

Counting Volume. A test with varying volumes of a solution containing a trace of carbon-14–sodium acetate in water-ethanol-toluene-diphenyloxazole showed that the counting volume of 35 ml. is not critical. The same specific activity was given by 30 and 35 ml. of radioactive solution; with 40 ml., the specific activity was 1.7% lower. It is true, however, that the carbon dioxide counting system might show a little greater sensitivity to errors in measuring the toluene or amine solutions because of the resultant changes in counting efficiency (Table II).

Precision. The standard deviation found for a set of nine similar samples of high activity processed through an entire operation was 0.34% of the mean value. This reproducibility compares favorably with other routine methods of measuring radioactivity.

Effect of Methanol. A solution of Hyamine base in methanol was prepared in the usual way; part of it was evaporated to give a 1*M* solution, then diluted with an equal volume of toluene. The two solutions were compared for counting efficiency at three levels of base: 1, 5, and 10 mmoles. The ratios of activities in methanol compared to methanol-toluene were 0.905, 0.795, and

0.382, respectively. Evidently there is considerable loss in counting efficiency at high carbon dioxide levels when part of the methanol is not removed.

Counting Substances Other Than Carbon Dioxide. Preliminary studies have shown that the hydroxide solution will dissolve large amounts of amino acids, mucic acid, hydrogen sulfide, and sulfur dioxide in toluene. This suggests the possibility of measuring radioactive sulfur by converting it to hydrogen sulfide or sulfur dioxide. The double flask has also been used for counting carbon-14 compounds by combusting the sample with silver nitrate and potassium persulfate (*5*) in one flask and collecting carbon dioxide directly in quaternary amine in the other flask. Details of this procedure are to be published.

Comparison with Other Counting Techniques. Carbon dioxide is ordinarily counted in the form of barium carbonate or carbon dioxide gas (*2, 7, 8*). The use of barium carbonate restricts sample sizes to a maximum of about 0.6 mmole. of carbon because of self-absorption. At the higher levels the efficiency is very low.

The techniques for counting gaseous carbon dioxide result in good efficiency, even with somewhat larger samples. The liquid scintillation method described here is similar to these techniques in sensitivity and permissible sample size. It appears to be superior to these procedures in that (1) the sample is readily stored after counting; (2) the carbon dioxide need not be purified before counting; (3) there is no need for liquid nitrogen and high-vacuum manifolds; and (4) intercomparison with other carbon-14 compounds can readily be made without burning the samples.

A method of scintillation counting with a sodium carbonate slurry that has been described briefly (*3*) may prove useful for somewhat larger amounts of carbon dioxide.

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Received for review October 21, 1955. Accepted January 6, 1956.

Particle Size Distribution and Number of Particles per Unit Mass of Some Fluorescent Powders

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Fluorescent fine particles were used as a tracer in the studies of washout of particulate matter by rain. The particle size distribution and number of particles per unit mass were determined by means of conventional methods, which appear to be unsatisfactory. New dilution-microscopic and dilution-photometric methods were developed. Equations for size distributions of different tracers were calculated. The results of analyses were compared and discussed. Whenever collected particles must be sized and counted, the dilution-microscopic method should be used to classify the tracer. If the size distribution of the sample is known, the rapid photometric method can be used to obtain the total number of particles.

DISPERSIONS of fluorescent particles present a convenient means of tracing the movement of air parcels over distances of many miles and of studying the behavior of aerosols under laboratory or field conditions.

Fluorescent tracers have been used by Perkins (*8*) in mesometeorological research and by Braham (*2*) in tracing of air particles to distances over 100 miles. In this laboratory fluorescent tracers have been used in a study of Langmuir's accretion theory under field conditions (*6*). Knowledge of the particle size distribution and of the number of particles per unit mass of tracer material is necessary for these studies.

In a study of dust washout in the free atmosphere, fluorescent particles (New Jersey Zinc, Inc., powders) were dispersed by a low-flying aircraft over a line of collectors resembling rain gages. Washed-out particles were collected by filtration from the rain, and the number of collected particles was determined. The total number of particles was estimated by area measurement (photometer readings of fluorescence). This method was satisfactory, providing the particle size distribution of the tracer as dispersed and the particle size distribution of the collected material were known. To minimize error in the determination of particle size distribution, the same method should be used both on the original tracer and on the collected sample.

The tracers used consist of a large number of single particles and a smaller number of aggregates and agglomerates. The number of particles per unit mass is obviously dependent upon a number of agglomerates originally present in a particulate matter. During experimentation some agglomerates are usually broken into single particles. The number of particles increases to a maximum when agglomerates are no longer present in the powder. Every particle or aggregate possesses a definite irregular shape which is nearly impossible to define in geometric terms. This fact leaves only statistical methods available for interpretation of size distribution of finely divided materials. The so-called "diameter" is used for defining the single dimension of the particle. The particle will have a different diameter at different orientations with respect to the linear graticule of the microscope. The diameter recorded was the length of the distance between two tangents on opposite sides of the apparent outline of the particle parallel to an arbitrary fixed direction at its random orientation. This is the so-called Feret's "statistical" diameter (*4*). This method requires a statistical number of measurements to eliminate the error in estimating the diameter from which the area may be derived. The shape factor was not introduced, because particles of needlelike shape or other odd shapes were not present in these tracers. This made it possible to choose the Feret's diameter for use in this study. The results of the microscopic examination are compared, when possible, with those of sedimentation methods.

To have representative numbers of particles, the microscopic counting and sizing were performed as follows: Heavily covered filters were evaluated by counting random fields; for less popu-

Table I. Number of Fluorescent Particles of Tracer per Pound

[Standard dilution method and microscopic technique, Whatman No. 54 (black) filter used]

	Tracer							
	NJZ 2330			NJZ 2210			NJZ 2205	
	Observer							
	1	2	3	1	2	3	1	2
	No. of Particles per Lb., $N \times 10^{-10}$							
	0.761	1.18	0.837	16.5	11.4	23.8	7.8	11.4
	0.956	1.08	0.830	12.9	9.3		13.2	9.3
	0.807	1.01	0.921	8.2	11.2		11.5	11.2
	1.03	0.93	0.656	12.5	9.0		9.45	9.0
		1.10	0.874					
		1.00						
Arithmetic mean	0.89	1.05	0.824	12.5	1.01	23.8[a]	10.5	10.2

[a] Whatman No. 50 filter (black) and photographic technique used. Value is arithmetic mean of 17 samples.

lated surfaces (between 3000 and 1500 particles per filter) a traversing technique was used; and for the least populated, the total filter surface was examined. For determination of the number of particles per unit weight and the number of particles in a certain size class per unit weight, a new method was investigated and developed.

EXPERIMENTAL

The procedure used was to weigh a small amount (approximately 0.1 gram) of dust prior to dispersing in a medium; an aliquot of this dispersion was diluted, and the second dispersion was sampled. The particles in this sample were counted to determine the number and size distribution of particles per unit mass.

The analytical method was established as follows: About 0.1 gram of fluorescent powder, dried at 110° C., was weighed in a dispersion vessel (wide-mouthed weighing bottle). A few drops of wetting agent (Aerosol OT) were added, and a paste was made and left for 5 minutes. The viscous solution (Karo sirup) was then added, the weight recorded, and a suspension prepared by complete mixing with a short, rubber-tipped glass rod. Stirring was continued for 10 minutes until a suspension, homogeneous in ultraviolet light, was obtained.

A new stirring rod was used to transfer about 1 gram of suspension into each of several weighing bottles, and about 30 grams of suspension medium was added to each bottle. This procedure was repeated until about 5×10^{-6} gram of dust was present in a single dilute suspension. The final dilute suspension was vacuum-filtered through a black (dyed with Sudan Black B) Whatman No. 50 filter paper and was washed with distilled water. The funnel, beaker, and stirring rod were checked carefully in darkness for fluorescent particles with an ultraviolet light. A dilute solution of poly(methyl methacrylate) was used to mount particles on the filter.

RESULTS

Phosphorescent powders NJZ 2210, 2205, and 2330 were examined. The dilution method as standardized above was used, and results are tabulated in Table I which show that the results of this method are consistent.

The size distribution of the particles was determined with the same dilution technique to prepare the sample for optical examination. Two sedimentation methods were used: the standard Andreasen pipet (*5*), and the sedimentation balance (*1*, *7*). The results of these experiments were plotted on log-probability paper and were nearly identical.

The photomicrographic technique used in connection with the dilution method consisted of taking pictures of all fluorescent particles under investigation. Because of different sizes of particles usually present in a field examined at random, up to nine negatives were prepared of each field to focus all sizes present. The results of the size distribution analysis are summarized in Table II.

The dilution-photometric method is based on the fact that the intensity of fluorescent light (measured as microamperes of phototube current) *vs.* the number of particles gives a straight line. In this way it is possible to determine rapidly the number of collected particles per standard filter. A tracer usually enters the experiment in a different size distribution from that collected after the experiment—e.g., large particles fall out, certain groups wash out. In addition to this the intensity of fluorescence often is changed. In such cases size distribution must be determined twice: before entering the experiment—e.g., after being dispersed by the feeding and dispersing mechanism (line *B*, Figure 1)—and after being collected in an experiment (line *A*, Figure 1)

Table II. Number Size Distribution of Tracers 2210, 2330, and 2205 by Different Optical Methods

	Tracer 2330, Photographic Method			Tracer 2210			Tracer 2205	
Diameter, μ	Observer 1	Observer 2	Arithmetic 3	Dilution photographic method[a], observer 1	Microscopic method[b], observer 2	Filar micrometer method, observer 3	Filar micrometer method, observer 1	Microscopic method, observer 3
0–1	...	...	...	17.34	28.0	...	...	13.0
1–2	...	...	...	36.24	40.0	...	...	41.0
2–3	...	...	...	19.87	18.0	...	...	19.0
3–4	...	...	...	8.56	7.5	...	...	11.0
4–5	...	...	...	5.90	3.5	...	...	9.0
0–5	18.02	20.04	19.03	87.91	97.0	...	...	93.0
5–6	...	...	...	4.60	1.4	4.24	3.06	3.0
6–7	...	...	...	2.48	0.7	3.16	3.79	2.2
7–8	...	...	...	1.51	...	2.33	3.22	0.8
8–9	...	...	...	0.86	..	1.77	2.01	0.4
9–10	...	...	...	0.83	..	1.11	1.69	0.2
5–10	17.26	15.08	16.17	10.28	3.0	12.61	13.77	6.60
7–10	...	...	...	...	0.9	...	...	...
10–15	16.12	16.86	16.49	...	..	...	...	...
15–20	12.53	12.77	12.65	...	..	...	...	...
20–25	12.18	10.90	11.54	...	..	...	...	...
10–25	...	...	...	1.80	..	...	...	0.4
25–30	7.87	9.99	8.93	...	..	...	...	...
30–35	5.08	4.96	5.02	...	..	...	...	...
35–40	3.82	3.22	3.52	...	..	...	...	...
40–45	2.16	1.88	2.02	...	..	...	...	...
45–50	1.52	1.60	1.56	...	..	...	...	...
50–100	3.44	2.70	3.07	...	..	...	...	...

[a] Arithmetic mean of 17 samples.
[b] Aggregates were broken into single particles.

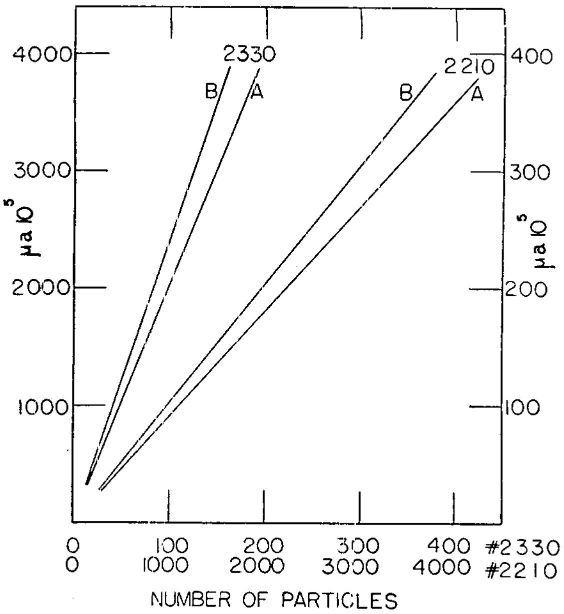

Figure 1. Relation between number of particles and intensity of fluorescent light for 2210 and 2330 tracers

***A*, after, and *B*, before an experiment**

For both analyses the same method should be used to eliminate the errors of the method, and often this is a very difficult task.

Logarithmic probability paper was used to plot the cumulative percentage *vs.* diameter. The equations representing the log-normal probability were derived in the form $y = a + bx$ where a is the logarithm of the corresponding mean diameter, and b is the logarithm of the standard deviation.

The equations are shown below for the following powders:

NJZ 2210
From photographic method $y_c^{2210} = 0.2932 + 0.3363\,u_p$ (1)

From microscopic method (aggregates not present) $y_c^{2210} = 0.1669 + 0.2876\,u_p$ (2)

NJZ 2205
From microscopic method $y_c^{2205} = 0.3016 + 0.2601\,u_p$ (3)

The results of sedimentation analyses were also plotted on logarithmic probability paper, and the equations derived are as follows:

NJZ 2210 $y_g^{2210} = 0.6070 + 0.3227\,u_p$ (4)

NJZ 2205 $y_g^{2205} = 0.8981 + 0.3468\,u_p$ (5)

NJZ 2266 (aggregates present) $y_g^{2266} = 0.6370 + 0.2721\,u_p$ (6)

(aggregates not present) $y_g^{2266} = 0.3019 + 0.2629\,u_p$ (7)

NJZ 2330 $y_g^{2330} = 1.360 + 0.2280\,u_p$ (8)

In the preceding equations $y_{c,g}$ (subscript c or g denotes the distribution obtained by count or by sedimentation, respectively) is equal to the logarithm of the corresponding diameter. The standardized cumulative normal distribution function is defined as:

$$\phi(u) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{u} e^{-x^2/2}dx$$

and the fractiles of the standardized normal variable are defined by the equation

$$p = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{u_p} e^{-x^2/2}dx, \quad u_{1-p} = -u_p$$

The median diameter by number or count is defined as the diameter for which 50% of particles are less than the stated size. The median diameter by weight gives equal masses on both sides of the stated size with completely different numbers of particles in each of the two groups.

In the equation $y = a + bu_p$ the logarithm of the median diameter is equal to a, because for the cumulative percentage of 50, the corresponding $u_p = u_{0.5}$, is equal to zero.

The standard deviation σ is equal to $\log^{-1} b$ or

$$\sigma = \frac{d_{84.13\%}}{d_{50\%}} = \frac{d_{50\%}}{d_{15.87\%}} = \frac{\log^{-1}(a + b)}{\log^{-1} a} = \frac{\log^{-1} a}{\log^{-1}(a - b)} = \log^{-1} b$$

To calculate the number of particles per unit mass, the mean volume diameter and the true density, ρ, of the powder must be known. The mean volume diameter is defined as:

$$d_v = \sqrt[3]{\frac{\Sigma nd^3}{\Sigma n}}$$

For the weight-in-size distributions which follow the log-probability distribution, the equations below permit transfer of size distribution from weight to a size basis (*3*).

$$\log d_v = \log d_g - 3.454 \log^2\sigma = a_g - 3.454\, b^2$$

$$\log d_v = \log d_c + 3.454 \log^2\sigma = a_c + 3.454\, b^2$$

where d_g is the median or geometric mean diameter by weight and d_c is the statistical diameter of the size frequency curve by count.

The number of particles per gram is then equal to

$$N/g = \frac{1}{0.5236\, d_v^3 \times \rho \times 10^{-12}}$$

where d_v is in microns and ρ is in grams per cubic centimeter.

The results of the calculation and direct counts are tabulated in Table III.

The numerical example is given below. The results of sedimentation analysis are for powder 2205. The equation of the straight line is derived by the least squares method.

Derivation

d, μ	Cumulative Fraction	Log d y_i	u_i	$y_i \times u_i$	u_i^2
0.92	0.013	−0.0339	− 2.226	+0.0755	4.9551
1.67	0.022	0.2227	− 2.014	−0.4485	4.0562
1.81	0.029	0.2577	− 1.896	−0.4886	3.5948
2.16	0.038	0.3344	− 1.774	−0.5932	3.1471
2.38	0.053	0.3766	− 1.616	−0.6086	2.6114
2.66	0.070	0.4249	− 1.476	−0.6271	2.1786
3.16	0.110	0.4997	− 1.226	−0.6126	1.5031
3.90	0.182	0.5911	− 0.907	−0.5361	0.8226
5.45	0.338	0.7364	− 0.418	−0.3078	0.1747
8.10	0.510	0.9085	0.025	+0.0227	0.0006
19.00	0.881	1.2787	1.180	+1.5089	1.3924
		5.5968	−12.348	−2.6154	24.4366

$$b = \frac{\Sigma(u_i \times y_i)\dfrac{(\Sigma u_i)(\Sigma y_i)}{n}}{u^2{}_i - \dfrac{(\Sigma u_i)^2}{n}} = 0.3468$$

$n = 11$

$$a_g = \bar{y}_i - b\bar{u}_i = 0.8981$$

$$y_g = a + bu_p = 0.8981 + 0.3468\, u_p$$

Calculation of different diameters based on sedimentation analysis (*3*) yields the following results:

Standard derivation, σ = antilogarithm of $b = 2.22$.

Mean volume diameter, $d_v = \sqrt[3]{\dfrac{\Sigma nd^3}{\Sigma n}}$

$$\log d_v = a_g - 3.454\, b^2 = 0.4826,\ d_v = 3.04 \text{ microns}$$

Table III. Number of Particles per Unit Mass by Different Techniques

Powder No.	Specific Gravity, G./Cc.	Method	Equation in Text No.	σ	d_v, μ	1 Gram	1 Lb.
2210	4.1	Dilution-photographic	1	2.17	4.83	4.13×10^9	1.87×10^{12}
		Dilution-photographic (direct count)	..	..	..	5.25×10^8	2.38×10^{11}
		Microscopic (agglomerates not present)	2	1.94	2.84	2.03×10^{10}	9.21×10^{12}
		Sedimentation	4	2.10	1.77	8.40×10^{10}	3.81×10^{13}
2330	4.1	Dilution-photographic (direct count)	..	..	..	2.03×10^7	9.20×10^9
		Sedimentation	8	1.69	15.2	1.33×10^8	6.03×10^{10}
2205	4.08	Microscopic	3	1.82	3.43	1.16×10^{10}	5.26×10^{12}
		Sedimentation	5	2.22	3.04	1.67×10^{10}	7.56×10^{12}
		Dilution (direct count)	..	..	..	2.27×10^8	1.03×10^{11}
2266	4.0	Sedimentation (agglomerates present)	6	1.87	2.41	3.41×10^{10}	1.55×10^{13}
		Sedimentation	7	1.83	1.15	3.09×10^{11}	1.40×10^{14}

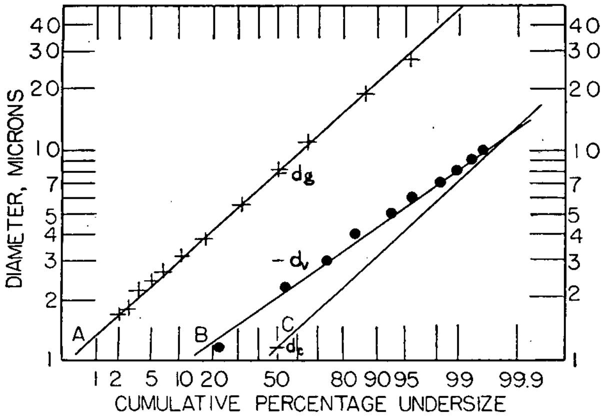

Figure 2. Cumulative size distribution of tracer 2205

A. Sedimentation method
B. Microscopic method
C. By count, derived from sedimentation analysis
Different diameters as derived from results of sedimentation analysis

Mass median diameter = geometric mean diameter (d_g):

$$\log d_g = a_g,\ d_g = 7.91 \text{ microns}$$

$$\text{Mean diameter by count, } d_c = \frac{\Sigma nd}{\Sigma n}$$

$$\log d_c = a_g - 6.908\, b^2 = 0.0671,\ d_c = 1.17 \text{ microns}$$

$$\text{Mean surface diameter, } d_s = \sqrt{\frac{\Sigma nd^2}{\Sigma n}}$$

$$\log d_s = a_g - 4.605\, b^2 = 0.3442,\ d_s = 2.21 \text{ microns}$$

The equation for the size distribution by count derived from sedimentation analysis,

$$y_c = 0.0671 + 0.3468\, u_p$$

Results of calculations are plotted in Figure 2.

DISCUSSION

An estimation of the number of particles per unit mass using the equations gives a higher number of particles than the direct count from the dilution method. Plots made on arithmetic or logarithmic probability paper represent size distributions asymptotic at both extremes. In reality, the investigated tracers represent normal distributions truncated on both sides. Truncation of the lower end of the curve (region of smaller diameters) has the largest influence on the calculated number of particles per unit mass.

Fluorescent tracer NJZ 2210 (Figure 3), with nearly all agglomerates broken into single particles, has a mean volume diameter of 2.84 microns and a standard deviation of 1.94 by microscopic examination. Sedimentation analysis gave d_v = 1.77 microns and σ = 2.10. This means that dispersion of the powder for sedimentation analysis also broke some of the agglomerates into single particles. This was confirmed by microscopic check. Calculation of the number of particles per unit of mass from the dilution method (direct count related to known mass of powder) gave 2.38 × 10^{11} particles per pound. When the same results (direct count and sizing) are used in the form of a plot on log-probability paper and by assuming that distribution is asymptotic (not one-sided truncated) the result of the calculation gives 1.87 × 10^{12} particles per pound—i.e., nearly eight times more. The mean volume diameter calculated from the size distribution obtained from photographs could not be compared in this case with others found from different methods because of image spread. The size distribution of the powder before and after the experiments was compared by means of identical techniques.

For No. 2330 the direct count gave 9.20 × 10^9 and for sedimentation 6.03 × 10^{10} particles per pound. The size distribution by the photographic method could not be easily expressed by an equation, because it gave a curve instead of a straight line on arithmetic or log-probability paper. In addition to this, there is a definite point of truncation around 3 microns (Figure 4).

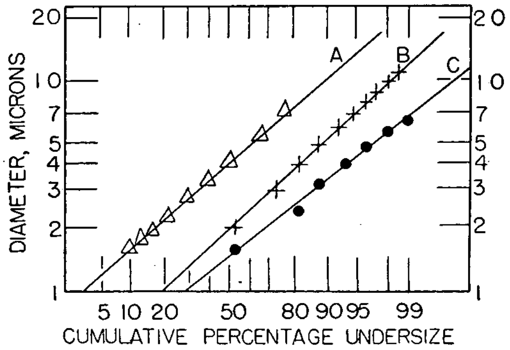

Figure 3. Cumulative size distribution of tracer 2210

A. Sedimentation analysis (Andreasen pipet)
B. Photomicrographic method
C. Microscopic method

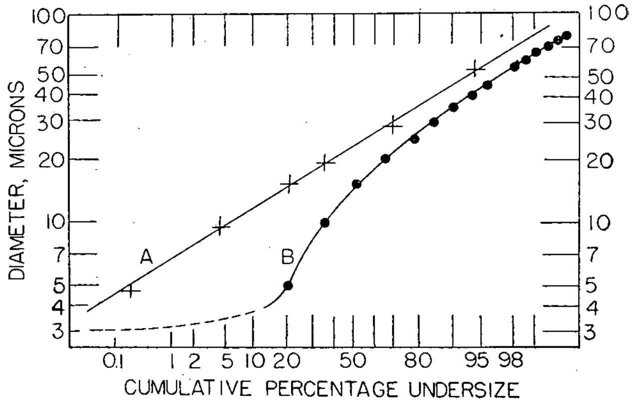

Figure 4. Cumulative size distribution of tracer 2330

A. Sedimentation analysis
B. Photomicrographic methods

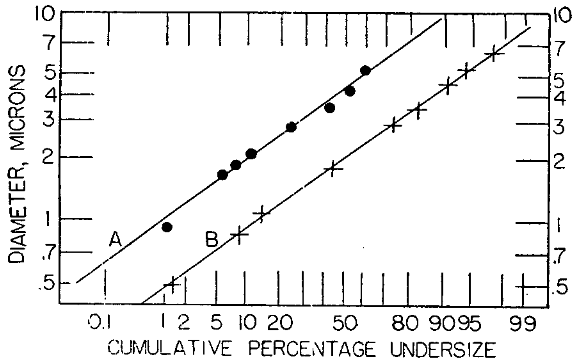

Figure 5. Cumulative size distribution of tracer 2266 obtained by sedimentation method (Andreasen pipet)

A. **Aggregates present**
B. **Aggregates broken**

The particles of No. 2205 dust are very close to spheres. This is the main reason that the results from calculations based on microscopic examination and sedimentation are in better agreement. The direct count (dilution method) gives a result of about $^1/_{60}$ the amount of the other results. This is easily foreseen because the size distribution is one-sided truncated at about 1 micron. The curve of microscopic count indicates 12% below 1 micron, whereas the count distribution curve derived by sedimentation indicates 42% less than 1 micron. This illustrates the necessity of the degree of truncation of the distribution when converting distribution from one basis to another (Figure 2).

Powder NJZ 2266 was used in qualitative experiments in high altitude cloud tracing. Because of the qualitative nature of the tests, only sedimentation analysis was used. Two suspensions were prepared, with and without agglomerates. From the results it is possible to see that, for all sizes, agglomeration is taking place within the powder resulting in a distribution with the same standard deviation but different mean volume diameter (Figure 5).

The results pertained here to the specific lots of powder used; however, limited investigation indicated only minor variation in both parameters.

ACKNOWLEDGMENT

This work was sponsored by the Air Force Cambridge Research Center, Geophysics Research Directorate, Contract No. AF 19(122)-472, C. E. Anderson, Scientific Officer. The assistance of M. A. Fisher, Supervisor of Fine Particles Research, Armour Research Foundation, in valuable discussions of particle size analysis and of Andrew Ungar in consultation and advice in the statistical field is gratefully acknowledged.

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Received for review September 1, 1955. Accepted January 17, 1956.

Determination of Six- and Seven-Carbon Naphthenes in Catalytic Reforming Feed

Report of the Subcommittee on Determination of Naphthenic Hydrocarbons, Committee on Analytical Research, Division of Refining, American Petroleum Institute

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Cooperative work in twelve laboratories has shown that there are several methods for accurate analysis of C_6 and C_7 naphthenes in reformer charge stock. Mass spectrometry, infrared spectrometry, refractivity intercept, and catalytic dehydrogenation methods have all been successfully used. In one California naphthenic charge stock, the individual C_6 naphthenes—cyclohexane and methylcyclopentane—have been determined with a standard deviation of only 0.5%. The C_7 naphthenes—methylcyclohexane and the sum of the six cyclopentane isomers—have been determined with a little less precision, but generally within 1% of the mean.

THE accurate determination of naphthenic hydrocarbons (cycloparaffins) has grown in importance to the petroleum industry with the expanding use of the catalytic reforming process. Naphthenes are converted to aromatics as the primary chemical reaction over various catalysts (*15*). By this means, the petrochemicals, benzene, toluene, and xylenes, and ethyl benzene, are produced in relatively large volumes from light naphtha fractions. High octane gasoline blending components are also made from virgin or straight-run distillates.

Good analytical methods have been available for aromatic hydrocarbons in gasoline range materials. At present, absolute accuracies from 2 to 3% down to 0.1 to 0.2% are available, depending upon what one can afford in manpower and elapsed

Table I. Boiling Points[a] of Individual C_5 to C_8 Naphthenes

No. of Carbons	Naphthene	Boiling Point ° C.	Boiling Point ° F.
5	Cyclopentane	49.3	120.7
6	Methylcyclopentane	71.8	161.3
	Cyclohexane	80.7	177.3
7	1,1-Dimethylcyclopentane	87.8	190.1
	trans-1,3-Dimethylcyclopentane	90.8	195.4
	cis-1,3-Dimethylcyclopentane	91.7	197.1
	trans-1,2-Dimethylcyclopentane	91.9	197.4
	cis-1,2-Dimethylcyclopentane	99.5	211.2
	Methylcyclohexane	100.9	213.7
	Ethylcyclopentane	103.5	218.2
8	Trimethylcyclopentanes (eight)	105–123	221–253
	Methylethylcyclopentanes (five)	121–128	249–262
	Isopropylcyclopentane	126.4	259.6
	n-Propylcyclopentane	130.9	267.7
	Dimethylcyclohexanes (seven)	119–130	247–266
	Ethylcyclohexane	131.8	269.2

[a] Data from (*1*).

time. Methods vary from those involving relatively simple equipment available in any laboratory to those involving large and expensive equipment and usually permitting economy in manpower. Considerably less attention has been paid to developing accurate methods for naphthenes.

In 1952, the American Petroleum Institute Committee on Analytical Research appointed a subcommittee for the comparison of methods of naphthene determination and development of new or improved methods where necessary. As its first assignment, the group undertook the comparison of methods in the C_6 and C_7 range. This was a logical starting point because more methods were available and greater accuracy was needed than in the higher boiling range.

Table I lists the various possible C_6 and C_7 naphthenes and their boiling points. Information is also given on the adjacent C_5 and C_8 naphthenes. There have been several reports (*5*, *8*, *19*) on the relative amounts of naphthenes in this boiling range covering a number of widely different crudes. The two major classes of naphthenes in the gasoline range are the cyclohexane (six-membered ring) derivatives and the cyclopentane (five-membered ring) derivatives. API Research Project 6 has shown that the relative proportions of these two classes differ among naphthas (*8*, *17*).

In reforming processes, benzene and toluene are produced from cyclohexane and methylcyclohexane by dehydrogenation, and from methylcyclopentane and seven-carbon cyclopentanes by isomerization and dehydrogenation. It is important to know the degree of conversion of naphthenes to aromatics by various catalytic reforming processes. Confronted with this problem, most petroleum analytical laboratories have felt the need for checking the accuracy of their naphthene methods.

The basic data and standard samples supplied by research projects sponsored by the American Petroleum Institute made possible the naphthene data and methods discussed in this paper. The spectroscopic methods are completely dependent upon calibrations made with API-NBS standard hydrocarbon samples supplied by API Research Project 6. The accurate physical property and spectroscopic correlations could not have been developed without the physical data and spectra on hydrocarbons provided by API Research Project 44 (*1*).

EXPERIMENTAL

Sample. A 170° to 220° F. charge stock from a California crude was chosen for the comparison of methods. Five drums of this particular stock were supplied by the California Research Corp., San Francisco, Calif. This material has been stored in a cold room by the Houdry Process Corp. at Linwood, Pa., and is still available for use in testing naphthene methods.

The following inspection data were obtained on the sample:

Gravity, °API	60.8
Aniline point, ° F.	115.3
ASTM D-86 distillation, ° F.	
Initial	162
5%	175
10%	177
50%	190
90%	212
End point	255

This sample proved to contain more than 85% C_6 and C_7 hydrocarbons. Less than 1% of the sample was lighter hydrocarbons and a little over 10% was heavier hydrocarbons.

There were 24 individual compounds in the C_6 and C_7 groups. Actually, the six dominant hydrocarbons consituted as much as 50% of the sample. Twelve hydrocarbons made up 75% of the sample. The over-all hydrocarbon type distribution was about 52% naphthenes, a little over 40% paraffins, and about 7% aromatics. The sample can be classed as a moderately high naphthene stock.

Data. Tables II through VII summarize the experimental data obtained on this sample by the 12 laboratories which participated in the study. All determined the C_6 naphthenes, 10 determined the C_7 naphthenes, and several determined the other naphthenes, the paraffins, and the aromatics as well.

Methods Used. Each laboratory was requested to use its best method, no matter how time-consuming this might be. The emphasis was placed upon referee-type methods to see whether different laboratories could agree under the most favorable conditions. Results by short-cut methods were also to be submitted where feasible.

Most of the laboratories chose to use either the mass spectrometer or the infrared spectrometer or a combination of the two. A few laboratories performed the detailed analyses using physical property correlations; two laboratories made use of Rampton's analytical dehydrogenation technique (*16*). Most of the short-cut analyses relied on the refractivity intercept correlation.

It is not the purpose of this paper to describe details of experimental methods. A brief discussion of the various procedures for the preliminary separations and for the determinations follows.

DEAROMATIZATION. Saturated hydrocarbon fractions can be more easily analyzed after the aromatic hydrocarbons are removed from the mixture. For use of physical property correlations, quantitative removal of the aromatics is essential. For infrared spectrometry, removal is preferable because it simplifies the absorption spectra. For mass spectrometry, there is very little interference of the aromatics with the saturates; some cooperators dearomatized the sample and others did not.

Aromatics were removed either by treating with acid, such as the sulfuric acid–phosphorus pentoxide mixture (*2*), or by adsorption on silica gel (*3*). Saturated hydrocarbon mixtures obtained from either type of processing appear to be satisfactory for further analysis.

DISTILLATION. Sharp separation of the hydrocarbon mixture into fractions containing a limited number of compounds was necessary for accurate analysis. Most laboratories used 3-, 4-, or 6-foot Podbielniak high-temperature distillation columns. Reflux ratios varied from 33 to 1 up to 100 to 1 and columns were operated at throughputs equivalent to 45 to 100 theoretical plates at total reflux.

A distillation curve for the saturates from this sample is shown in Figure 1. In this example a 200-ml. sample was distilled in a Podbielniak column 3 feet × 13 mm. at a throughput of 330 ml. per hour and a 45 to 1 reflux ratio. An aromatic hydrocarbon chaser was used. The boiling points of the predominant saturated hydrocarbons in the California naphtha are shown in Figure 1. Among the 12 laboratories the number of fractions taken depended on the method of analysis and varied from three or four up to 30 or more.

MASS SPECTROMETRY. Mass spectra are useful for determining either individual hydrocarbons or groups of similar hydrocarbons (*6*, *7*). For individual hydrocarbons, it is best to work with distillation fractions narrow enough to contain only one isomeric group for any given type of hydrocarbon. For example, accurate determination of cyclohexane and methylcyclohexane in the

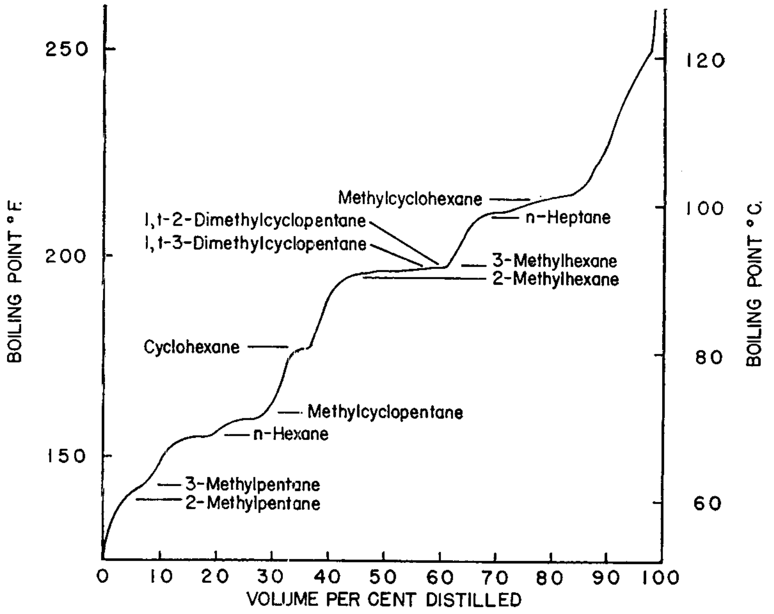

Figure 1. Distillation curve for naphthene-paraffin portion from California naphthenic charge stock

same fraction would be difficult because of interference by other components at the significant mass numbers. Determination of individual close-boiling isomers such as some of the dimethylcyclopentanes is almost impossible.

For group analysis, correlations are derived based on summations over certain specific mass numbers. For example, cyclohexane and cyclopentane derivatives can be calculated separately by using different mass peaks (*9*). The sum of the dimethylcyclopentanes is easily determined even though the individual compounds may not be determined.

Laboratories B, J, K, and M analyzed the entire C_6 and C_7 range for naphthenes and paraffins using four to eight fractions. In analyzing the C_6 range alone, two or three fractions were sufficient.

INFRARED SPECTROMETRY. Infrared spectrometry has been used for analyzing saturated hydrocarbon mixtures for a number of years (*5*, *20*). The infrared absorption spectrum of each individual hydrocarbon is unique. Therefore, in principle every compound can be determined. However, there are many absorption bands in the spectrum of a C_6 or C_7 hydrocarbon, and overlapping of bands in mixtures is a problem. For accurate analysis, it is preferable to have mixtures containing no more than seven to 10 compounds.

The infrared determination of groups of saturated hydrocarbons such as cyclohexane derivatives or cyclopentane derivatives has not generally been done. There are few, if any, absorption bands specific for each group.

Laboratories H, L, and N analyzed the C_6 and C_7 range for all individual naphthenes and paraffins. Twenty to 30 fractions or blends were analyzed.

Several laboratories combined infrared and mass spectrometry for the determination of the C_6 naphthenes, thus making use of the advantages of each method.

PHYSICAL PROPERTY CORRELATIONS AND DEHYDROGENATION. Accurate determinations of individual saturated hydrocarbons in various different naphthas have been made by API Research Project 6 (*8*). Through knowledge of the boiling points of pure hydrocarbons and the wide differences in refractive indices of paraffins and naphthenes, *individual* compounds were quantitatively determined in fractions obtained by distillation at 200 theoretical plates. Physical property correlations are more frequently applied to the determination of groups of similar hydrocarbons than to the determination of individual compounds.

Functions of refractive index and density such as refractivity intercept (*12*, *13*, *21*, *22*) and specific refraction (*16*) are used for determining proportions of paraffins and naphthenes. Special correlation charts must be used for each fraction when highest accuracy is desired (*10*, *22*). The two different types of naphthenes—namely, cyclohexanes and cyclopentanes—are not separately determined by such physical property correlations except where they can be first separated by distillation.

An auxiliary chemical method for determining cyclohexane derivatives is the catalytic dehydrogenation technique of Rampton (*14*, *16*). Cyclohexanes are converted to aromatics, which are determined in the product. Cyclopentanes remain unchanged and are calculated as the difference between total naphthenes and total cyclohexanes.

Laboratories A, F, L, and M determined the C_6 and C_7 naphthenes by various combinations of physical property correlations and other methods. Two of them determined the cyclohexanes by dehydrogenation and one by infrared spec-

Table II. Determination of Individual C_6 Naphthenes

Laboratory	Volume % of Whole Sample		Method[a] of Analyzing Fractions
	Methyl-cyclopentane	Cyclohexane	
A	9.3	6.5	Spec. refraction
B	10.6	7.0	MS
C	10.3	6.4	IR, MS
E	10.2	6.0	MS
F	10.0	5.7	$(n-d/2)$, IR
G	9.4	7.6	MS
	10.8	6.8	IR, MS
H	10.4	5.9	IR
J	10.5	6.3	MS
K	9.6	6.3	MS
L	9.5	6.4	IR
	10.5	5.5	$(n-d/2)$, dehydrogenation
M	10.4	6.1	MS
	10.6	6.3	$(n-d/2)$
N	10.4	6.2	IR
	10.6	6.1	IR, MS
Av.	10.2	6.3	
Std. dev.[b]	0.5	0.5	
Max. dev.	0.9	1.3	

[a] MS is mass spectrometer, IR is infrared spectrometer, $(n-d/2)$ is refractivity intercept.

[b] Standard deviation $= \sqrt{\frac{\Sigma\,(\text{av.} - \text{exp. value})^2}{(n-1)}}$

Table III. Determination of Individual C_7 Naphthenes

Laboratory	Volume % of Whole Sample								Method[a] of Analyzing Fractions
	Dimethylcyclopentanes					Ethyl cyclopentane	Total C_7 cyclopentanes	Methylcyclohexane	
	1,1	*trans*-1,3-	*cis*-1,3-	*trans*-1,2-	*cis*-1,2-				
A							19.5	10.9	Spec. refraction, dehydrogenation
B	2.2	←13.1→				0.8	16.1	11.7	MS
E	←15.2→					0.4	15.6	11.4	MS
F							18	9.2[b]	$(n-d/2)$, IR
H	1.1	1.7	..	7.6	..	0.8	11.2[b]	12.1	IR
J	1.4	←8.0→		←6.6→		1.1	17.1	11.8	MS
K	1.7	←9.5→		3.5	0.8	0.6	16.1	11.3	MS
L	1.8	3.0	2.7	5.9	0.7	1.0	15.1	11.9	IR
							17	11.5	$(n-d/2)$, dehydrogenation
M	1.8	←5.7→		6.6	1.8	0.9	16.8	11.6	MS
	←14.6→				←13.8→				$(n-d/2)$
N	1.2	4.6	2.1	5.4	1.6	1.0	15.9	11.7	IR
Av.	1.6	3.1	2.4	5.8	1.2	0.9	16.7	11.6	
Std. dev.	0.4	Insufficient data				0.2	1.3	0.3	
Max. dev.	0.6	1.5	0.3	2.3	0.6	0.5	2.8	0.7	

[a] MS is mass spectrometer, IR is infrared spectrometer, $(n-d/2)$ is refractivity intercept.

[b] Omitted from calculation of average and deviations because values are outside 95% confidence limit.

trometry. Three to five fractions were analyzed to determine cyclohexane, methylcyclohexane, methylcyclopentane, and total C_7 cyclopentanes.

DISCUSSION

C_6 Naphthenes. The individual determinations of cyclohexane and methylcyclopentane are listed in Table II and plotted in Figure 2. Excellent agreement was obtained among the 12 laboratories. Considering that there were many different methods used and no concerted attempt had been made to standardize the procedures, such good agreement was surprising. The methylcyclopentane content averaged 10.2% by volume of the sample and the cyclohexane content 6.3% by volume. Standard deviations in both cases were only 0.5%; the maximum deviation of any determination from the mean value was only a little over 1%.

The scattering of data about the average values in Figure 2 shows that there was no dependence upon the type of method. Apparently, any of the methods is satisfactory as long as the laboratory is familiar with the method and has good calibrations.

C_7 Naphthenes. The data on C_7 naphthenes are shown in Table III. Methylcyclohexane was the hydrocarbon in highest concentration in the whole naphtha. The average value was 11.6%. This compound was determined by all the different methods with as good accuracy as the two C_6 naphthenes.

The six cyclopentane isomers were more difficult to determine. Three of the dimethylcyclopentanes are close-boiling and only one isomer was in a concentration of more than 3%. Infrared is probably the best method for determining each individual compound. For many purposes the sum of these compounds is all that is desired; methods such as mass spectrometry and the physical property–dehydrogenation combination are then adequate. More than half the results are within 1% of the 16.7% average value for the total C_7 cyclopentanes.

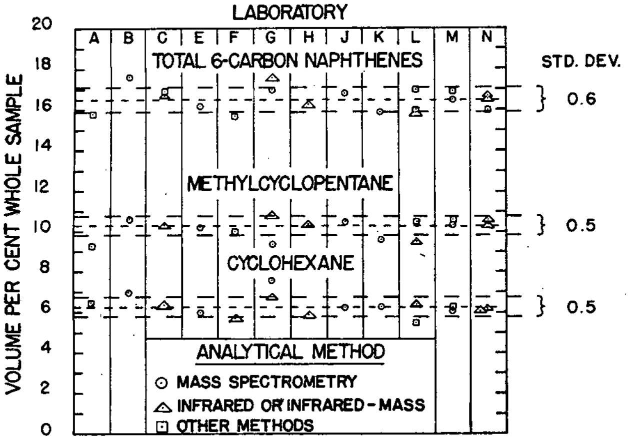

Figure 2. Six-carbon naphthenes in California naphthenic charge stock

Total Naphthenes. Results on total naphthenes are summarized in Table IV. The data for the C_6 and C_7 isomeric groups are the sums of the data in Tables II and III. Six-carbon naphthenes, averaging 16.5%, were determined with a standard deviation of only 0.6%. Seven-carbon naphthenes, averaging 28%, were determined with less certainty, but only two of the 12 values differed from the mean by more than 1%. Approximately 44% of the sample is C_6 and C_7 naphthenes.

Several laboratories which completely analyzed the sample found a few tenths of 1% of cyclopentane in this naphtha and about 7% of C_8 naphthenes. The best value for total naphthene content of the sample, obtained by summing the individual compounds and groups, is 52 ± 1%.

Detailed naphthene determinations such as reported here are expensive. Estimates of time consumed in these analyses ranged from 7 man-hours for the C_6 naphthenes, using the mass spectrometer, to 100 man-hours for the individual naphthenes and paraffins by infrared spectrometer. (This includes time for distillation or preparation of the sample.) No effort, of course, had been made to reduce these analyses to a routine basis. The mass spectrometric method is the most rapid, but also requires the most expensive equipment. The infrared method is the most time-consuming and gives the most detailed information. The physical property–dehydrogenation type of method is between the two extremes.

Short-Cut Methods for Total Naphthenes. Most laboratories also have to determine total naphthene contents of naphthas on a routine basis. Table V shows data obtained by some routine methods on this sample. Most of these short-cut analyses were done by use of refractivity intercept charts on the whole dearomatized sample or on distillation cuts. Laboratory B, however, carried out the total naphthene analysis by mass spectrometry and Laboratory H determined the individual C_6 naphthenes by a short-cut infrared method (*11*).

All the data agree well with the more detailed analyses. This is very encouraging and probably means that these laboratories have done a careful job of deriving their correlation charts and selecting distillation cut points. These methods are satisfactory for the C_6 and C_7 range, but need further checking in the range of 8 carbons and higher. These short-cut methods also need checking in concentration ranges other than 50% naphthenes. This subcommittee and Section F of ASTM D-2 Research Division IV expect to do more work along this line.

Table IV. Determination of Isomeric Groups

Laboratory	Volume % Naphthenes in Whole Sample					
	C_5	C_6	C_7	C_8	C_6 and C_7	C_5 to C_8
A	0.7	15.8	30.4	4.3	46.2	51.2
B	0.2	17.6	27.8	8.0	45.4	53.6
C		16.7				
E	0.3	16.2	27.0	9.3	43.2	52.8
F	0.3	15.7	27	6	42.7	49
G		17.0				
		17.6				
H	0.3	16.3	23.3[a]		39.6[a]	
J	0.2	16.8	28.9	6.3	45.7	52
K		15.9	27.4	9.7	43.3	53
L	0.2	15.9	27.0		42.9	
		16.0	28.5	7.5	44.5	52
M	0.3	16.5	28.4		44.9	
	0.6	16.9	28.4	8.0	45.3	53.9
N		16.6	27.6			
		16.7				
Av.	0.3	16.5	28.0	7.4	44.4	52.2
Std. dev.	0.2	0.6	1.0	1.8	1.3	1.6
Max. dev.	0.4	1.1	2.4	3.1	1.8	3.2

[a] Omitted from calculation of average and deviations because values are outside 95% confidence limits.

Table V. Short-Cut Naphthene Determinations

Laboratory	Volume % Naphthenes in Whole Sample				
	Total	C_5	C_6	C_7	C_8
	Refractivity Intercept on Distillate[a] Saturate Fractions				
C	..	0.5	16.9	28.1	..
F	49	0.3	15.7	27	6
L	52		17	28	7
N	51.3	0.4	16.0	29.9	5.0
	Refractivity Intercept on Whole Saturates				
C	50				
E	52				
F	50				
L	51				
	Mass Spectrometer, Whole Sample				
B	55				
	Infrared[b]				
H			16.3[c]		
Av.	51	0.4	16.4	28.2	6
Av. from Table IV	52.2	0.3	16.5	28.0	7.4

[a] Cut points must be carefully selected in this sort of analysis to get good breaks between isomeric groups. Each laboratory chose its cut points.

[b] Method described by (*11, 18*).

[c] Individual C_6 isomers: methylcyclopentane, 10.0%; cyclohexane, 6.3%.

Table VI. Determination of Individual Paraffins

	Volume % of Whole Sample										
	C_5	C_6				C_7				C_8	
B.P., ° C.	*n*-Pentane 36	*n*-Hexane 69	2- and 3-Methylpentanes 60–63	Others 50–58	Total	*n*-Heptane 98	2- and 3-Methylhexanes 90–92	Others 79–94	Total	Total	Method[a] for Analyzing Cuts
Laboratory											
B	0.2	8.4	9.6	0.6	18.6				16.7	3.8	MS
E	0.2	8.4	9.7	0.8	18.9				17.0		MS
H	0.2	9.1	9.1	1.0	19.2	8.6	9.5	3.6	21.7		IR
J	0.1	8.6	9.0	0.6	18.2	8.0	6.9	3.2	18.1		MS
K		10.2	7.4	..	17.6	8.5	4.8	4.3	17.6	4.1	MS
L	0.2	8.6	9.4	0.5	18.5	8.3	7.6	2.7	18.6		IR
M	..	8.4	8.8	..	17.2	7.7	7.7	2.2	17.6		MS
		7.9	←—8.8—→		16.7	8.2	←—9.6—→		17.8	4.7	$(n-d/2)$
N		8.9	9.4	0.6	18.9	7.2	8.3	2.6	18.1		IR
Av.	0.2	8.7	9.0[b]	0.7[c]	18.2	8.1	7.5[d]	3.1[e]	18.1	4.2	
Std. dev.	0	0.6	0.7	0.2	0.9	0.5	1.6	0.8	1.5	..	
Max. dev.	0.1	1.5	1.6	0.3	1.5	0.9	2.7	1.2	3.6	0.5	

[a] MS is mass spectrometer, IR is infrared spectrometer, $(n-d/2)$ is refractivity intercept.
[b] About 4.9% 2-methylpentane, 4.2% 3-methylpentane.
[c] About 0.6% 2,3-dimethylbutane, 0.1% 2,2-dimethylbutane.
[d] About 3.2% 2-methylhexane, 4.3% 3-methylhexane.
[e] About 1.7% 2,3-dimethylpentane; 0.6% 2,4-dimethylpentane; 0.4% 3-ethylpentane; 0.3% 2,2-dimethylpentane; 0.2% 3,3-dimethylpentane.

Table VII. Determination of Individual Aromatics

	Volume % of Whole Sample						
B.P., ° C.	Benzene 80	Toluene 111	Total C_6 and C_7	C_8 136–144	Total C_6, C_7, C_8	Total aromatics[a]	Method of Analysis[b]
Laboratory							
B	2.4	4.4	6.8	0.2	7.0	7.6	MS
C						7	(*4*)
E	2.3	4.8	7.1	0.2	7.3	7.0	MS
G						6.8	(*2*, *4*)
H	1.3	4.6	5.9				IR
J	2.2	4.0	6.2			7.3	(*3*, *4*) MS
K	2.3	4.6	6.9				MS
L						7.6	(*2*)
M						7.0	(*4*)
N	2.1	4.6	6.7				Spec. dispersion
Av.	2.1	4.5	6.6	0.2	7.2	7.2	
Std. dev.	0.4	0.3	0.5	..	..	0.2	
Max. dev.	0.8	0.5	0.7	..	0.2	0.4	

[a] As determined on whole sample.
[b] MS is mass spectrometer, IR is infrared; (*2*), (*3*), and (*4*) refer to cited ASTM methods: acid absorption, silica gel adsorption, and fluorescent-indicator-adsorption, respectively.

Paraffins and Aromatics. Tables VI and VII summarize the other data obtained incidentally during this program. Good agreement was obtained between laboratories on major components such as *n*-hexane, *n*-heptane, the four singly branched paraffins, benzene, and toluene. These data also show good agreement among a variety of methods.

DISPOSITION OF SAMPLE

A large portion of the sample remains in storage for use by any petroleum laboratory for evaluating naphthene methods for the C_6 and C_7 range. It may be particularly useful for checking short-cut methods which are used on a routine basis. Currently an ASTM group seeking to standardize mass spectrometric methods for hydrocarbon-type analysis of gasolines is using this material as one of its cooperative samples.

CONCLUSIONS

This work has shown that there are several good methods for determining C_6 and C_7 naphthenes within 1% in reforming charge stocks. Each method has certain inherent advantages. Naphthene analyses can be obtained by individual compounds or by groups.

Any detailed naphthene analysis requires fractional distillation. Removal of aromatics prior to the analysis of the saturates is necessary for some methods and optional for others. Mass spectrometry is the most rapid method for determining naphthenes in some detail. Infrared spectrometry is the best method for determining all individual hydrocarbons. For laboratories which do not need the detailed analyses or do not have the necessary equipment, accurate naphthene determinations can be obtained using relatively simple physical measurements, such as density and refractive index. The chemical method involving dehydrogenation of cyclohexanes to aromatics can also be used in such laboratories.

The subcommittee concludes that there is no need for further cooperative testing in the C_6 and C_7 range. There are satisfactory referee methods for comparison with more rapid methods in the individual laboratories. The subcommittee is continuing with a program on methods for determining total naphthenes in whole gasoline.

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Retention Volumes of Isomeric Hexenes and Hexanes in Gas Liquid Partition Chromatography Using Phthalate Esters as Liquid Phase

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By gas partition chromatography the retention volumes for the isomeric hexenes and hexanes have been determined on a column of given dimensions, using a thermal conductivity cell for detection and a 10-mv. range strip-chart recorder. Liquid substrates were di-*n*-decylphthalate and ditetrahydrofurfurylphthalate impregnated on Celite 545, with nitrogen gas as the carrier. Comparisons are given for the retention volumes (pressure corrected) of the olefins on both substrates using several combinations of temperature and flow rate. The corrected retention volumes for the isomeric hexanes at one temperature and flow rate used for the olefins have been determined and are compared with the olefins. Retention volumes for all the isomers are also given relative to *n*-pentane, because this compound may be used as an internal standard.

IN RECENT years several reports on the use of gas partition chromatography as an analytical tool have appeared in the literature. Separations of hydrocarbons (*8, 10*); aldehydes, ethers, and alcohols (*10*); fatty acids (*4, 5*); esters (*2, 10*); amines (*3, 6*); and mixed systems (*7*) have been described. With proper operating conditions almost any material with an appreciable vapor pressure can be handled by this method.

James and Martin (*4, 5*) give a theory for the partition column. The present work was undertaken to study some of the applications of this method and theory to a difficult analytical problem, such as the separation of the 17 isomeric hexenes with a boiling range of only 32° C. The isomeric hexanes were also examined because it was expected that, if some of the close boiling olefin pairs could not be distinguished, their hydrogenation products could be separated.

THEORY

James and Martin in a theory for gas partition chromatography have set forth the basic equation for the retention volume of a component of a mixture as

$$V_R = \frac{2aL}{3R_f}\left[\frac{(P_1/P_0)^3 - 1}{(P_1/P_0)^2 - 1}\right] \qquad (1)$$

where V_R is the volume of carrier gas necessary to remove the concentration maximum of a given component from the column; a is the area occupied by the gas phase in any cross section of the column; L is the length of the separating section; R_f is the ratio of the movement of the zone of maximum concentration of the component to the movement of the carrier gas (the ratio of linear velocities); P_1 is the pressure of the carrier gas at the column inlet; and P_0 is the pressure of the gas at the column exit. The retention volume V_R is dependent on the ratio, $[(P_1/P_0)^3 - 1]/[(P_1/P_0)^2 - 1]$, for a given column and temperature.

James and Martin have shown that a limiting value for the retention volume can be calculated from

$$V_R^\circ = 3/2\ V_R\left[\frac{(P_1/P_0)^2 - 1}{(P_1/P_0)^3 - 1}\right] \qquad (2)$$

To calculate V_R°, the terms on the right-hand side of Equation 2 must be evaluated. P_1 and P_0, the inlet and exit pressures, are readily measured. V_R, the retention volume, is the product of the flow from the exit of the column and the time necessary to remove the maximum concentration zone of a component.

For routine application of this technique, it is often more convenient to determine V_R° relative to some internal standard. If Equations 1 and 2 are expressed as ratios for Components 1 and 2, the operating and column variables disappear. From this the identity is found that

$$\frac{(V_R^\circ)_1}{(V_R^\circ)_2} = \frac{(V_R)_1}{(V_R)_2} = \frac{(R_f)_2}{(R_f)_1}$$

If, however, any of the operating variables are changed, the proper correction factor must be inserted into the equation. This may, but does not necessarily, pertain to a change in substrate.

EXPERIMENTAL

The apparatus is a modification of that of Patton, Lewis and Kaye (*9*). It is shown diagrammatically in Figure 1. Exit pressure was taken as barometric pressure.

This system differs from that of Patton and others in the use of twin columns. Without a reference column it was found that pressure fluctuations caused both an instantaneous and a delayed detector response with resulting base line instability. The modified system increases the stability of the base line, because flow variations are nearly in phase.

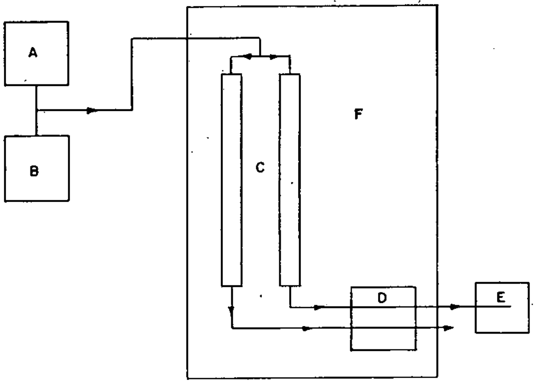

Figure 1. Schematic diagram of apparatus

A. Controlled pressure gas source
B. Differential manometer
C. Reference and measuring columns, 0.5 cm. in inside diameter and 110 cm. long. These are packed to give nearly equal flow rates.
D. Double arm thermal conductivity cell (*7*)
E. Wet test meter
F. Controlled temperature bath

The stationary phases consisted of 40% by weight of di-*n*-decylphthalate (Eastman Chemical 6447-P) for the first substrate, and ditetrahydrofurfurylphthalate (Eastman Chemical 3048-P) for the second substrate. Both were impregnated on diatomaceous earth (Johns Mansville Celite No. 545). The carrier gas used for both series was nitrogen. The hexanes were Phillips Pure Grade.

The experimental plan was divided into four parts:

A. To test the reproducibility of Equation 2, retention volumes for the 17 isomeric olefins were determined at each of two (P_1/P_0) values. In all experiments *n*-pentane was run as a reference standard before and after each olefin.

B. Retention volumes were measured at each of two temperatures to determine the effect of temperature.

C. The olefins were also run on the second substrate (ditetrahydrofurfurylphthalate) and the retention volumes determined at one temperature and one flow rate.

D. The retention volumes of the isomeric hexanes were determined on both substrates at one temperature and flow rate.

Sample size in all cases was approximately 10 µl. Samples were introduced into the column through a rubber serum cap by means of a hypodermic syringe.

RESULTS

In Table I the isomeric hexenes and hexanes are listed separately in the order of increasing boiling point at 760 mm. of mercury (*1*). The hexane to which each hexene is hydrogenated is also shown. The spread in boiling point is 32° C. for the 17 hexenes and 19° C. for the hexanes. For the hexenes, with exception of the highest and lowest boiling isomers and their first neighbors, the maximum difference in boiling point between neighboring pairs is 2.8° C. and the average difference is only 1.2° C.

In the following discussion, the hexenes and hexanes are referred to by the numbers shown in Table I. In the other tables and the figures, all retention volumes are the limiting values, V_R°, for the column used as calculated from Equation 2. Different values would be obtained for a column of different dimensions.

In Table II are given the retention volumes and relative retention volumes ($V_R^\circ{}_{\text{olefin}}/V_R^\circ{}_{n\text{-pentane}}$) for the hexenes as determined using the di-*n*-decylphthalate substrate. In column 1, with the exception of three olefin pairs (4 and 5, 9 and 10, 13 and 14), the average retention volumes for the two flow rates follow the same order as the boiling points. The retention volumes calculated from Equation 2 give good agreement for the two flow rates, the average deviation from the mean for the 17 olefins being less than ±3%, which is the deviation found for repacking the column. Relative retention volumes for the two flow rates (column 2) show the same agreement as the retention volumes.

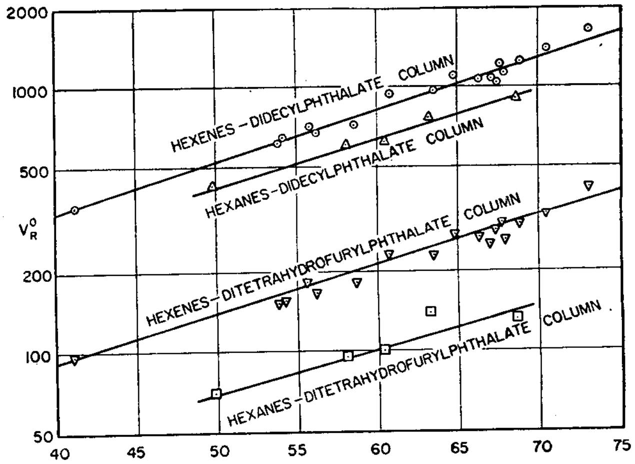

Figure 2. Logarithm of retention volumes (V_R°) at 20° C. with respect to boiling point for hexenes and hexanes

In columns 3 and 4 of Table II are found the retention volumes and relative retention volumes for a flow rate of 24 ml. per minute, but at a column temperature of 25° C. Comparison of columns 1 and 2 with columns 3 and 4 shows that the higher temperature gives lower values for the retention volumes; but there is almost no change in relative retention volumes for the two temperatures. The percentage change in V_R° for the temperature interval of 5° C. is 20% on the average. The maximum change is 26% for 3-methyl-*cis*-2-pentene, while the minimum change is 16% for 3,3-dimethyl-1-butene.

Table III gives the retention volumes for the olefins and the retention volumes relative to *n*-pentane determined on a ditetrahydrofurfurylphthalate column at a flow of 27 ml. per minute at 20° C. This is the same column operated at the same pressure ratio used for the di-*n*-decylphthalate substrate. The retention volumes in each case are lower by a factor of almost four than those determined for the di-*n*-decylphthalate column. The results otherwise are very nearly identical for the two substrates, as may be seen by comparing the relative retention volumes for the ditetrahydrofurfurylphthalate substrate at 20° C. with the relative retention volume for the di-*n*-decylphthalate substrate at the same temperature. The average change in relative retention volumes was 3%, which is within experimental error.

Table I. Hexenes and Hexanes in Order of Increasing Boiling Point

No.	Hexene	Boiling Point, ° C.	Hydrogenation Product
1	3,3-Dimethyl-1-butene	41.2	2,2-Dimethylbutane
2	4-Methyl-1-pentene	53.9	2-Methylpentane
3	3-Methyl-1-pentene	54.1	3-Methylpentane
4	2,3-Dimethyl-1-butene	55.7	2,3-Dimethylbutane
5	4-Methyl-*cis*-2-pentene	56.3	2-Methylpentane
6	4-Methyl-*trans*-2-pentene	58.6	2-Methylpentane
7	2-Methyl-1-pentene	60.7	2-Methylpentane
8	1-Hexene	63.5	*n*-Hexane
9	2-Ethyl-1-butene	64.7	3-Methylpentane
10	*cis*-3-Hexene	66.4	*n*-Hexane
11	*trans*-3-Hexene	67.1	*n*-Hexane
12	2-Methyl-2-pentene	67.3	2-Methylpentane
13	3-Methyl-*trans*-2-pentene	67.6	3-Methylpentane
14	*trans*-2-Hexene	67.9	*n*-Hexane
15	*cis*-2-Hexene	68.8	*n*-Hexane
16	3-Methyl-*cis*-2-pentene	70.5	3-Methylpentane
17	2,3-Dimethyl-2-butene	73.2	2,3-Dimethylbutane
	Hexane		
1	2,2-Dimethylbutane	49.7	
2	2,3-Dimethylbutane	58.0	
3	2-Methylpentane	60.3	
4	3-Methylpentane	63.3	
5	*n*-Hexane	68.7	

Table II. Retention Volumes for Hexenes Using Di-*n*-decylphthalate Column

		1		2		3	4
		Retention Volume, V_R°, 20° C. Flow Rate		Relative Retention Volume, $V_R^\circ{}_{\text{olefin}}/V_R^\circ{}_{n\text{-pentane}}$, 20° C. Flow Rate		Retention Volume, V_R°	Relative Retention Volume, $V_R^\circ{}_{\text{olefin}}/V_R^\circ{}_{n\text{-pentane}}$
No.	Hexenes in Order of Increasing Boiling Point	24 ml./min.	43 ml./min.	24 ml./min.	43 ml./min.	25° C.; Flow Rate, 24 Ml./Min.	
1	3,3-Dimethyl-1-butene	365	370	1.20	1.20	305	1.20
2	4-Methyl-1-pentene	625	625	2.05	2.00	515	2.05
3	3-Methyl-1-pentene	650	630	2.10	2.05	520	2.05
4	2,3-Dimethyl-1-butene	730	710	2.40	2.30	575	2.30
5	4-Methyl-*cis*-2-pentene	690	705	2.25	2.30	560	2.25
6	4-Methyl-*trans*-2-pentene	730	720	2.40	2.30	580	2.30
7	2-Methyl-1-pentene	945	940	3.10	3.05	770	3.05
8	1-Hexene	975	920	3.20	3.00	775	3.10
9	2-Ethyl-1-butene	1110	1120	3.60	3.65	865	3.45
10	*cis*-3-Hexene	1080	1100	3.50	3.55	890	3.50
11	*trans*-3-Hexene	1090	1170	3.50	3.80	875	3.45
12	2-Methyl-2-pentene	1160	1170	3.85	3.80	940	3.70
13	3-Methyl-*trans*-2-pentene	1230	1270	4.00	4.15	975	3.90
14	*trans*-2-Hexene	1140	1130	3.70	3.65	920	3.60
15	*cis*-2-Hexene	1250	1250	4.10	4.00	1000	3.95
16	3-Methyl-*cis*-2-pentene	1390	1410	4.55	4.55	1080	4.30
17	2,3-Dimethyl-2-butene	1640	1640	5.40	5.35	1260	5.05

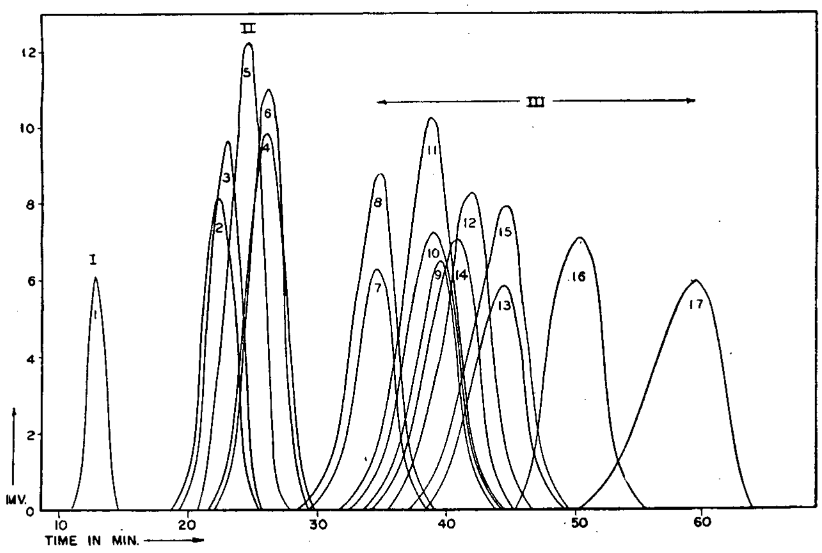

Figure 3. Composite drawing of experimental curves of hexenes on di-*n*-decylphthalate column at 20° C.

Table III. Retention Volumes for Hexenes Using Ditetrahydrofurfurylphthalate Column

No.	Hexenes in Order of Increasing Boiling Point	Flow Rate, 27 Ml. per Minute at 20° C. Retention volume, V_R°, ml.	Relative retention volume, V_R° olefin/V_R° n-pentane
1	3,3-Dimethyl-1-butene	95	1.30
2	4-Methyl-1-pentene	155	2.15
3	3-Methyl-1-pentene	155	2.15
4	2,3-Dimethyl-1-butene	180	2.55
5	4-Methyl-*cis*-2-pentene	170	2.35
6	4-Methyl-*trans*-2-pentene	180	2.50
7	2-Methyl-1-pentene	230	3.20
8	1-Hexene	230	3.20
9	2-Ethyl-1-butene	275	3.80
10	*cis*-3-Hexene	270	3.75
11	*trans*-3-Hexene	255	3.55
12	2-Methyl-2-pentene	290	4.00
13	3-Methyl-*trans*-2-pentene	305	4.25
14	*trans*-2-Hexene	260	3.65
15	*cis*-2-Hexene	300	4.20
16	3-Methyl-*cis*-2-pentene	335	4.65
17	2,3-Dimethyl-2-butene	415	5.75

The retention volumes of the hexanes using the two substrates are given in Table IV. These values were determined at a flow rate of approximately 27 ml. per minute and a temperature of 20° C. The retention volumes and the relative retention volumes for the hexanes follow the order of increasing boiling point except for the inversion of 3-methylpentane and *n*-hexane on the ditetrahydrofurfurylphthalate substrate.

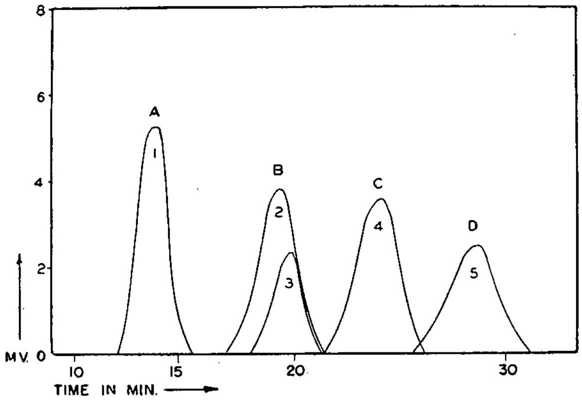

Figure 4. Composite drawing of experimental curves of hexanes on di-*n*-decylphthalate column at 20° C.

DISCUSSION OF THE RESULTS

The logarithms of the retention volumes (limiting value) given in the tables for the olefins and hexanes for 20° C. are plotted in Figure 2 as a function of boiling point. In general, within a class the logarithm of the retention volume is a linear function of boiling point for both substrates used. For individual cases, however, some deviation from exact linearity is found. The slopes are very nearly equal for the substrates used in this study. The di-*n*-decylphthalate column is preferable because it gives time differences between olefin pairs greater by a factor of four over the time differences for the ditetrahydrofurfurylphthalate column. However, because the hexanes are different by a factor of six on the two substrates, the ditetrahydrofurfurylphthalate column might have some advantage in certain cases.

In Figure 3 is a composite drawing of the curves for all 17 of the hexenes. The numbers within the peaks correspond to the number in Table I. The olefins within the composite fall into three groupings designated by Roman numerals I, II, and III in the figure. Group I consists of a single olefin and is readily identified. Group II contains olefins which can be identified only as a unit, because the thermal conductivity detector would produce a nearly symmetrical composite or additive peak. Group III would be observed as a main peak, including nine olefins, and two shoulders. The shoulders consist of olefins 16 and 17, which could be identified if present in significant amounts. Thus, for a sample containing equal amounts of the 17 olefins, five groups can be identified by a single run of the mixture.

Table IV. Retention Volumes for Hexanes

(27 ml. per minute flow rate at 20° C.)

No.	Hexanes in Order of Increasing Boiling Point	V_R°, Ml. Col. 1[a]	V_R°, Ml. Col. 2[b]	V_R° hexane/V_R° n-pentane Col. 1[a]	V_R° hexane/V_R° n-pentane Col. 2[b]
1	2,2-Dimethylbutane	430	70	1.45	1.15
2	2,3-Dimethylbutane	620	100	2.10	1.65
3	2-Methylpentane	635	100	2.15	1.65
4	3-Methylpentane	770	140	2.60	2.30
5	*n*-Hexane	920	135	3.10	2.20

[a] Di-*n*-decylphthalate substrate.
[b] Ditetrahydrofurfurylphthalate substrate.

From any mixture of Group II, it would not be possible to distinguish whether a peak represented a single olefin or a mixture. For olefins 9, 10, 11, 12, and 14, some information might be obtained if all of the olefins were not present in significant amounts. In such a case the olefin pair 7 and 8 could be distinguished from the pair 13 and 15, if the bulk group 9, 10, 11, 12, and 14 were missing or only present in very small amount.

Thus, from a single run of a mixture, olefins 1, 16, and 17 could be identified, and in certain cases olefin pairs 7 and 8 and 13 and 15 could be distinguished as pairs.

A composite drawing of the curves for hexanes is given in Figure 4. Of the four peaks, only *B* represents more than one component. Thus three of the hexanes are distinguishable and the other two occur as a pair.

If a hexene fraction is run on a column and olefin groupings

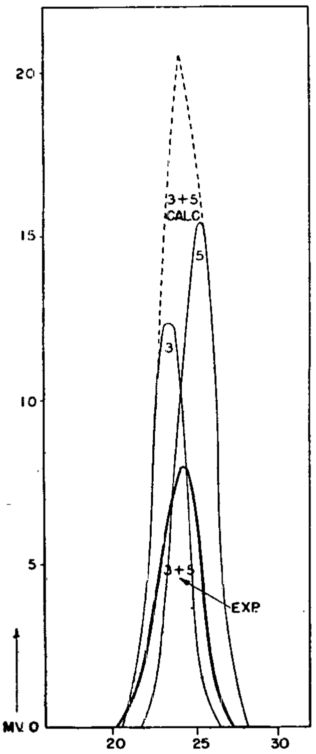

Figure 5. Experimental and calculated curves for olefins 3 and 5 on di-*n*-decylphthalate column at 20° C.

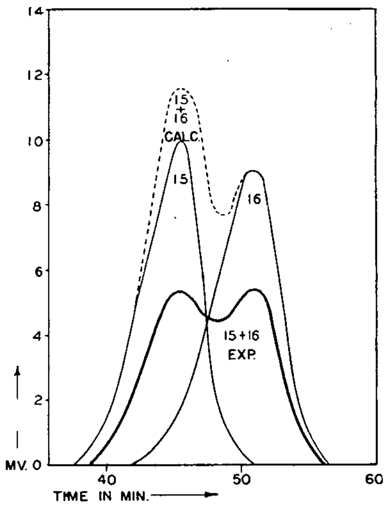

Figure 6. Experimental and calculated curves for olefins 15 and 16 on di-*n*-decylphthalate column at 20° C.

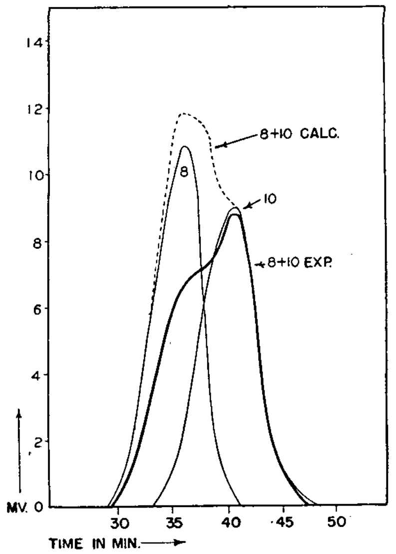

Figure 7. Experimental and calculated curves for olefins 8 and 10 on di-*n*-decylphthalate column at 20° C.

are identified, hydrogenation and running as hexanes could be used to give additional information. Some examples of how this information can be used are illustrated in Figures 5 to 7.

In Figure 5 are shown the individual pure olefin determinations for olefins 3 and 5 from Table I, together with the summed or calculated curve and the experimentally determined curve for the mixture of the pair. From this figure it is found that the two olefins give a single peak as predicted by adding the individual curves. Thus, identification cannot be made, other than that they belong to Group II (Figure 3). Hydrogenation of these olefins gives 3-methylpentane and 2-methylpentane. Figure 4 shows that these hexanes give two distinct peaks, 4 and 3, respectively. In Group II, however, there remain three other olefins, 2, 4, and 6. Numbers 2 and 6 give 2-methylpentane (3 in Figure 4), while number 4 gives 2,3-dimethylbutane (2 in Figure 4). Thus, from the example given, the presence or absence of one olefin can be established. By this technique the other portion of the mixture cannot be characterized beyond a possible one or all of four olefins. Another substrate, a longer column, or an additional analytical technique would be needed to resolve them.

In Figure 6 are the experimental curves for the individual olefins 15 and 16, a calculated composite, and the curve for the mixture, which is considered to be an unknown. Comparison of the experimental curve in Figure 6 with Figure 3 shows that the sample mixture consists of Group III olefins. From Figure 3 it is apparent that this mixture consists of 16 and possibly 9, 10, 11, 12, 13, 14, and 15. Because of symmetry, it can also be shown that olefins 9, 10, 11, and 14 could be present only in small amounts. Hydrogenation of the mixture gives 3-methylpentane (4 in Figure 4) for 16. Olefins 9 and 13 also give 3-methylpentane, while 10, 11, 14, and 15 give *n*-hexane (5 in Figure 4), and 12 gives 2-methylpentane (3 in Figure 4). Hydrogenation of this sample, therefore, gives a mixture of 3-methylpentane and *n*-hexane, a separable mixture by Figure 4. The mixture is then olefin 16 and possibly olefins 9, 10, 11, 13, 14, and 15. However, if quantitative samples of both hexenes and hydrogenated products were used, a comparison of areas under the curves makes it apparent that 9 and 13 cannot be present in quantities greater than the error in measurement of areas. Also, considerations of symmetry show that olefin 15 is the major remaining component.

The importance of symmetry consideration is brought out in Figure 7, which includes single experimental determinations on olefins 8 and 10, the calculated composite curve, and an experimental curve. This figure shows that the shape of the curve for a mixture and the position of the maximum may vary with composition, apparently producing peak shifts. This means that an asymmetric peak or a shifted peak indicates two or more components, or some other interfering phenomenon.

CONCLUSIONS

Complete analysis of a complex mixture of narrow boiling range is not likely to be achieved by any single method. However, much useful information can be obtained by the technique of gas partition chromatography. A combination of this method with other chromatographic techniques and other analytical processes should furnish sufficient data to characterize qualitatively even as complex a mixture as the isomeric hexenes.

ACKNOWLEDGMENT

The hexenes were furnished through the courtesy of K. W. Greenlee, American Petroleum Institute Research Project 45, Cecil E. Boord, director, a project of Ohio State University Research Foundation.

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RECEIVED for review October 14, 1955. Accepted December 29, 1955. Division of Petroleum Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September, 1955.

Thermogravimetric Pyrolysis of 8-Quinolinol and 5,7-Dihalo-8-quinolinol Chelates of Scandium, Thorium, and Uranium(VI)

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The thermogravimetric pyrolysis of the 8-quinolinol, 5,7-dichloro-8-quinolinol, and 5,7-dibromo-8-quinolinol chelates of scandium, thorium, and uranium(VI) was determined. It was found that only two of the chelates can lose the extra molecule of solvation by thermal decomposition—namely, the 8-quinolinol chelates of thorium and uranium(VI). Oxide levels of the ignited chelates were found in the temperature range from 450° to 600° C. The sublimation curves for the three chelating agents were also investigated.

SCANDIUM, thorium, and uranium(VI) form chelates with 8-quinolinol and the 5,7-dihalo-8-quinolinols having the general formula, $M(Ox)_nHOx$, where M is the metal ion of oxidation number *n* and HOx represents the chelating molecule. Contrary to the normal chelates of 8-quinolinol with metal ions (*1*), these particular compounds contain an extra molecule of solvation. For the scandium chelate, this extra molecule is thought to be held by weak lattice forces in the solid state and is said to be of the order of magnitude of about 1 kcal. per mole (*18*).

Because of the favorable gravimetric factors of the 8-quinolinol chelates, they have been employed for the determination of scandium (*16*), thorium (*9*), and uranium(VI) (*3, 6, 8, 10*). However, there is some question as to the temperature limits for drying the precipitated chelates. Frere (*7*) reported that the thorium 8-quinolinol chelate was stable up to 100° to 110° C., but lost the extra molecule of solvation between 160° and 170° C. Above this temperature the chelate decomposed rapidly. Using the Chevenard thermobalance, Dupuis and Duval (*4*) found that there was no loss in weight to a constant composition in the temperature range from 20° to 945° C. Using the same experimental method, Borrell and Pâris (*2*) found that the anhydrous chelate containing the extra molecule of solvation was stable up to about 80° C., decomposed by an intermolecular dehydration above 137° and then lost the extra molecule above 245° C. On further heating, the thorium oxide level was obtained at a higher undesignated temperature.

The uranium 8-quinolinol chelate loses the extra solvate molecule easily at 210° to 215° C. (*15*). Duval (*5*) found that the chelate began to lose 8-quinolinol at 157° and formed the normal chelate at 252° C. Above 346° the normal chelate began to decompose, but the oxide, U_3O_8, was not obtained even at 947° C.

The scandium 8-quinolinol chelate has not been studied on the thermobalance, but, from thermal decomposition at temperatures up to 165° C., it was found that the extra molecule of solvation could not be removed without total decomposition of the chelate (*18*).

The 5,7-dichloro-8-quinolinol and 5,7-dibromo-8-quinolinol chelates of scandium (*14*), thorium (*12*), and uranium(VI) (*13*) have recently been investigated. It was reported that the extra molecule of solvation could not be removed by thermal decomposition. However, under carefully controlled conditions, chelates could be prepared which did not contain the solvate molecule.

Because of the gross inconsistencies in the literature regarding the thermal decomposition of the 8-quinolinol chelates of thorium and uranium(VI), and the new series of the 5,7-dihalo-8-quinolinol chelates, it was decided to study the chelates on a new type of thermobalance (*19*). It was of interest to determine if the extra solvate molecule could be thermally removed in the 5,7-dihalo-8-quinolinol chelates and how these chelates compare in stability with the unhalogenated quinolinol chelates. The lack of an oxide level in the uranium(VI)- and thorium 8-quinolinol chelates (*4, 5*) at elevated temperatures also warranted further experimental study.

EXPERIMENTAL

Reagents. 8-Quinolinol (melting point, 74–6° C.) and 5,7-dibromo-8-quinolinol (melting point, 195–6° C.) were obtained from the Matheson, Coleman and Bell Co., East Rutherford, N. J. 5,7-Dichloro-8-quinolinol (melting point, 177–9° C.) was obtained from the Eastman Organic Chemicals Co., Rochester 3, N. Y. They were used without further purification.

Scandium oxide of 99.8% purity was obtained from A. D. Mackay, Inc., New York, N. Y.; thorium nitrate tetrahydrate was obtained from the Lindsay Chemical Co., West Chicago, Ill.; and uranyl nitrate hexahydrate was obtained from Merck & Co., Inc., Rahway, N. J.

All other reagents were of C.P. quality.

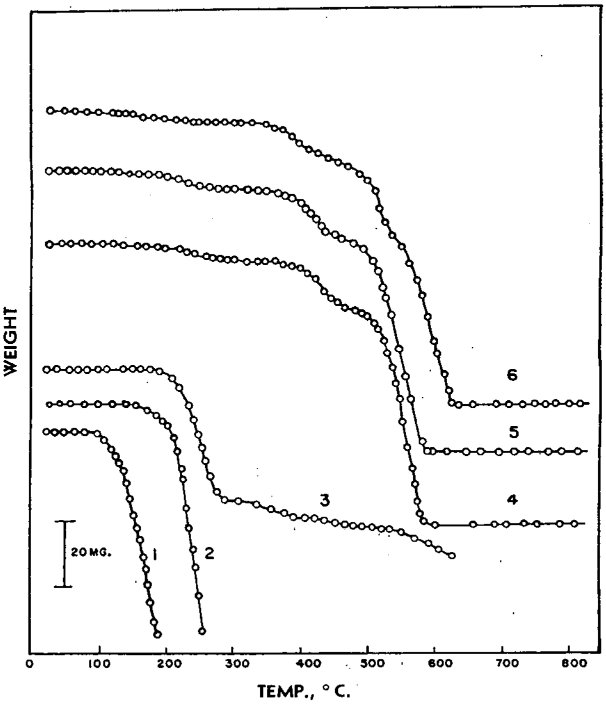

Figure 1. Pyrolysis curves of chelating reagents and scandium chelates

1. 8-Quinolinol
2. 5,7-Dichloro-8-quinolinol
3. 5,7-Dibromo-8-quinolinol
4. Scandium-8-quinolinol chelate
5. Scandium 5,7-dichloro-8-quinolinol chelate
6. Scandium 5,7-dibromo-8-quinolinol chelate

Thermobalance. The thermobalance used has previously been described (*19*).

Preparation of Chelates. The 8-quinolinol chelates of scandium (*17*), thorium (*11*), and uranium(VI) (*15*) were prepared by methods previously described. The 5,7-dihalo-8-quinolinol chelates of scandium (*14*), thorium (*12*), and uranium(VI) (*13*) were also prepared by methods previously described.

All of the precipitated chelates were air dried at room temperature (25° to 27° C.) for 24 hours before thermogravimetric pyrolysis on the thermobalance.

DISCUSSION

8-Quinolinol and 5,7-Dihalo-8-quinolinols. Although primary interest was in the metal chelates of the three compounds, the volatility of the pure materials was first determined. The sublimation curves for 8-quinolinol, 5,7-dichloro-8-quinolinol, and 5,7-dibromo-8-quinolinol are shown in Figure 1. 8-Quinolinol is the most volatile (atmospheric pressure about 680 mm.). The first weight loss occurred at 85° C.; the rate of weight loss became rapid between 100° and 200° C. Thus it is possible to remove an excess of this reagent by heating the precipitates above 85°.

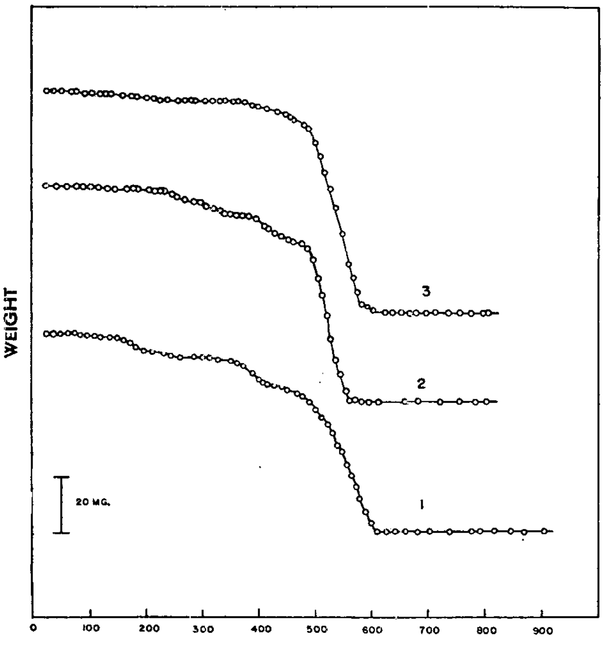

Figure 2. Pyrolysis curves of thorium chelates

1. Thorium 8-quinolinol chelate
2. Thorium 5,7-dichloro-8-quinolinol chelate
3. Thorium 5,7-dibromo-8-quinolinol chelate

The 5,7-dihalo-8-quinolinols show both sublimation and decomposition. The dichloro- derivative began to sublime at 142° C., increasing to a very rapid rate of sublimation between 200° and 250° C. There was no decomposition of the compound. The dibromo- derivative began to sublime at 175° C., but decomposition followed after two thirds of the material was removed by sublimation. At 290° a black tar remained in the thermobalance pan; the tar was gradually oxidized as the temperature increased to 600° C. Obviously, the dibromo- derivative is the least stable.

Scandium Chelates. The pyrolysis of the 8-quinolinol, 5,7-dichloro-8-quinolinol, and 5,7-dibromo-8-quinolinol chelates of scandium is shown in Figure 1. From an over-all view, the decomposition curves are of the same general pattern. An interesting aspect of the three curves is that an oxide level corresponding to Sc_2O_3 was found in all three cases. The 8-quinolinol chelate was the most stable, with the first weight loss occurring at 125° C. The least stable was the 5,7-dibromo-8-quinolinol chelate, which began to lose weight at 80° C. In no case did a constant weight level occur which had the stoichiometry for the normal chelate, $Sc(C_9H_6NO)_3$. Rather, a mixture of the solvated chelate, $Sc(C_9H_6NO)_3 . C_9H_6NOH$, and the normal chelate was formed until the oxidation of the organic material began at 350° to 400° C. The oxide levels were obtained at about 600° C. for all three of the chelates. Of course, in the analytical determination using the 8-quinolinol chelate, it is impractical to ignite to the oxide because of the favorable gravimetric factor for the chelate, 7.24% scandium. However, the maximum temperature limit for drying the chelate is 125° C. The pyrolysis of the pure 8-quinolinol (Figure 1), shows that the reagent began to sublime at 85° C., so that any temperature between 85° and 125° would be satisfactory for drying. The 5,7-dihalo-8-quinolinol chelates are not recommended for the determination of scandium from the standpoint of thermal stability and also because of the difficulty in preparing the pure chelate in the desired stoichiometry.

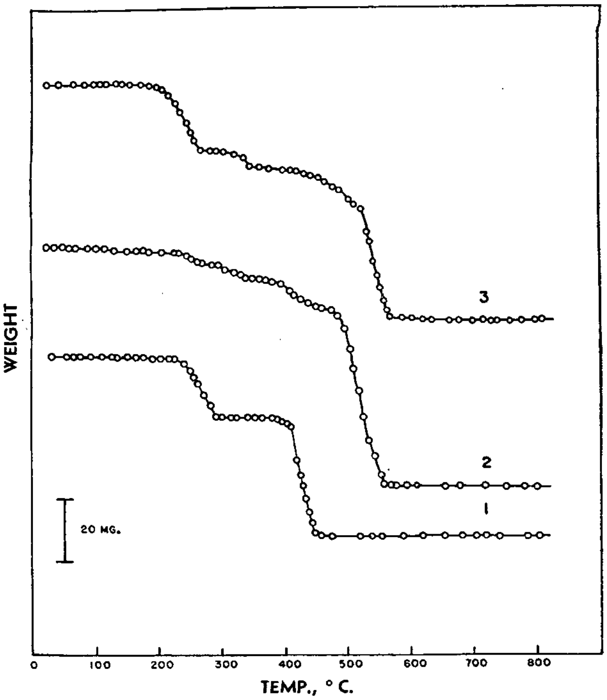

Figure 3. Pyrolysis curves of uranium chelates

1. Uranium 8-quinolinol chelate
2. Uranium 5,7-dichloro-8-quinolinol chelate
3. Uranium 5,7-dibromo-8-quinolinol chelate

Thorium Chelates. The pyrolysis of the three chelates of thorium is shown in Figure 2. The results obtained with the 8-quinolinol chelate agree with those of Borrell and Pâris (*2*). The chelate was stable up to 80° C., where it began to lose 8-quinolinol. A constant weight level resulted at 250° C., which corresponded to the normal chelate, $Th(C_9H_6NO)_4$. The normal chelate began to decompose at 310° C., resulting in an oxide level of ThO_2 beginning at 600° C. The results differ considerably from those of Dupuis and Duval (*4*), in which an oxide level was not found even at 945° C. The reason for the disagreement between the two previous workers using the same type of thermobalance is not known.

The 5,7-dihalo-8-quinolinol chelates are about as stable as the 8-quinolinol compound. In no case was a region of constant weight obtained which would designate the normal chelates; therefore, it appears to be impossible to prepare them in this manner. There seems to be no advantage in using the dihalo chelates for the determination of thorium, except possibly a better gravimetric factor. The difficulties in precipitation would, however, more than compensate for the difference.

Uranium Chelates. The pyrolysis of the three chelates of uranium(VI) is shown in Figure 3. In the case of the 8-quinolinol chelate, $UO_2(C_9H_6NO)_2 . C_9H_6NOH$, the decomposition proceeded smoothly to the normal chelate, $UO(C_9H_6NO)_2$, beginning at about 230° C. This higher temperature for the sublimation of 8-quinolinol from the chelate indicates some degree of interaction in the solid state. As stated previously, 8-quinolinol begins to sublime at 85° C. However, when held as a molecule of solvation, it sublimes at 230° C. The results seem to indicate that the lattice forces holding this extra molecule of 8-quinolinol must be of a greater order of magnitude than 1 kcal. per mole.

The normal chelate was stable up to 380° C., where it decomposed to the oxide level, U_3O_8, beginning at 450° C. This is certainly in disagreement with Duval (*5*) who did not obtain an oxide level even at 947° C.

Although it is possible to prepare the normal chelate of uranium and 8-quinolinol by thermal decomposition, it is not possible to remove the extra solvate molecule of the 5,7-dihalo-8-quinolinol chelates by heating. There was no evidence from the pyrolysis curves for the existence of the normal chelates in this series of compounds. The 5,7-dihalo chelates were even less stable thermally than the 8-quinolinol chelate; thus, there is no advantage in using them for the determination of uranium. It is difficult to prevent coprecipitation in the chelates, as is shown by the decomposition of the uranium 5,7-dibromo-8-quinolinol chelate in Figure 3. The excess reagent began to sublime at about 200° C. going directly to the chelate, $UO_2(C_9H_4Br_2NO)_2 \cdot C_9H_4Br_2NOH$.

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Received for review November 15, 1955. Accepted December 30, 1955

Color Reaction of Amino Acids with Alloxan, Isatin, and Ninhydrin in Circular Paper Chromatography

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Color reactions of 51 amino acids and their sensitivities were determined as spot tests on filter paper with alloxan, isatin, and ninhydrin. R_f values for these amino acids were obtained with the circular chromatographic method using phenol– and butanol–acetic acid as developing solvents. Alloxan gave stable and uniform colors for most amino acids and was a more sensitive reagent than ninhydrin for some of them. For amino acids with similar R_f values, simultaneous use of these reagents applied to sectors from one chromatogram often permits the "differential" determination of a single amino acid in a mixture. Isatin is the most useful for this purpose because of the wide variety of its color reactions and lack of reactivity with many amino acids. The nature of these color reactions for amino acids and their possible application to amino acid mixtures are discussed.

TWO general approaches have been tried for the complete qualitative identification of amino acids in complex mixtures using paper chromatography. The first approach aims at complete resolution of amino acids into bands or spots containing but one component. A single, generally applicable coloring reagent would then suffice for the identification of all the amino acids present. However, complete resolution is difficult to achieve in practice. An alternative approach is the use of specific or selective coloring reagents to identify a single amino acid in a band or spot containing several components. Attempts to obtain more complete separation of the individual amino acids in a mixture have led to such developments as: (*a*) increased path of travel of components by using larger paper sheets (*48*) or ascending-descending chromatography (*5, 48*); (*b*) the use of buffered filter paper (*26, 30*); (*c*) the use of multiple solvent systems (*14, 19, 21, 30, 37–39*); (*d*) multiple development techniques (*11, 23*); and (*e*) elution from one chromatogram followed by rerunning the mixture on a second chromatogram employing a different solvent system (*19*). It is the opinion of Block, Durrum, and Zweig (*6*) that many of these inovations represent no major improvement over the two-dimensional, two-solvent technique originally described in the classical paper by Consden, Gordon, and Martin (*9*). Indeed, many of these modifications have made the procedure more costly, complex, and laborious.

One exception to this general trend appears to be the increasing popularity of the circular chromatographic method proposed by Brown (*8*) and Rutter (*41, 42*). In this method the substances to be analyzed are resolved into circular bands instead of spots. Recent work by LeStrange (*25*), as well as previous studies by Müller and Clegg (*33*), have confirmed the simplicity of the method, as well as the facts that separation occurs more rapidly, and the definition between components is superior to that obtained with the descending technique. Careful experimental studies of some of the physical factors which influence circular paper chromatography were made by Saifer and Oreskes (*43*). These studies have led Block and others (*6*) to conclude that circular paper chromatography is a more rapid and a simpler method than the more conventional techniques.

In addition to the advantages stated above, circular paper chromatography lends itself most readily to the use of several different color reagents, because a large number of sectors can be cut from a single chromatogram (*41, 42*). Such an approach permits the identification of the separate amino acids even where complete resolution has not occurred (*17*). The use of such differential reagents which give colors with one or several amino acids has often been cited in the literature (*4, 6, 17, 18, 48*). Although ninhydrin comes closest to the ideal coloring reagent because of its reactivity and sensitivity for most amino acids, even it has certain drawbacks, such as the faint yellow colors obtained with proline and hydroxyproline (*9, 23*). For this reason isatin was proposed as an additional color reagent, because it gives intense bluish colors with these two imino acids (*2*). Subsequent studies (*44*) showed that a number of other amino acids also react with this reagent, thus enabling it to be used for their identification. In a recent publication (*34*) a scheme was proposed for the complete identification of the amino acids of protein hydrolyzates, except for separation of the leucines. This system employs two solvents (phenol-water and butanol–acetic acid) and two coloring reagents (ninhydrin and isatin).

The present paper deals with an extension of this system to other amino acids besides those previously discussed (*34*). It also makes use of alloxan as a useful reagent for amino acids in paper chromatography to supplement the ninhydrin and isatin reactions. Although the reactivity of alloxan with amino acids in general has been noted in the literature (*1*, *10*, *22*, *27*), only one previous application to the field of paper chromatography of amino acids and related compounds was found (*40*). It is believed that the data presented here will be useful in identifying the less common amino acids such as may be found in urine, plant and tissue extracts, protein hydrolyzates, and the like (*7*, *16*, *52*).

EXPERIMENTAL

Reagents. NINHYDRIN, reagent grade, 0.25% in reagent grade acetone. This reagent remains stable over a period of several months.

ISATIN, reagent grade, 0.2% in reagent grade acetone containing 4% acetic acid. This reagent is also stable for several months. A slight precipitate which forms occasionally should be filtered off prior to use.

ALLOXAN, reagent grade, 0.25% in reagent grade acetone. When the solution is first made, any undissolved material should be filtered off. This reagent is relatively unstable; it should be made fresh weekly and kept refrigerated.

ISOPROPYL ALCOHOL, reagent grade.

Procedure for Determining Reactivity and Sensitivity of Various Amino Acids. Solutions of 5 mmoles per liter of the various amino acids shown in Table I were prepared by dissolving the pure amino acid in the appropriate volume of 10% isopropyl alcohol. Whatman No. 1 filter paper was used, and 20 cu. mm. of each amino acid were spread uniformly over an area within a penciled circle 1 cm. in diameter. This operation was performed on three separate sheets of filter paper, one for each coloring reagent to be tested. Each sheet was dipped into one of the coloring reagents, air dried, and then heated at about 100° C. for 10 minutes. If the reaction was positive, the above procedure was repeated with smaller aliquots of the same solution—e.g., 15, 10, and 5 cu. mm. At this point, if the reaction was still positive, a 1 to 5 dilution of the amino acid was made, and the procedure again repeated. This was continued until no perceptible reaction was noted. The next higher concentration was then taken as the limit of sensitivity for the particular amino acid with each coloring reagent. These values, as well as the colors obtained, are given in Table I.

Determination of R_f Values by Circular Paper Chromatography. The R_f values for the various amino acids listed in Table I were determined with the circular chromatographic technique, using phenol-water (0.1% ammonia) and butanol–acetic acid–water (40 to 10 to 50) as the developing solvents, exactly as described in previous publications (*34*, *43*). The R_f values, as shown in Table I, represent the average values for at least six separate runs for each developing solvent. In most instances ninhydrin was used as the coloring reagent to show the exact location of the circular band in relation to the solvent front. The R_f values for each chromatogram were calculated in the manner previously described (*43*). In other cases, where ninhydrin was less sensitive or reacted poorly, alloxan or isatin was used. In every case care was taken to remove even faint traces of the developing solvents, either by acetone washing in the case of phenol, or by air drying with a stream of warm air in the case of the butanol–acetic acid. This was necessary to prevent any interference with the subsequent color reactions and to make shades of color obtained on the actual chromatogram comparable with those obtained with the spotting technique.

Table I. Circular Paper Chromatographic R_f Values

[Phenol-water (0.1% ammonia) and butanol–acetic acid–water (40 to 10 to 50) used as developing solvents. Minimum detectable quantities given for various amino acids with ninhydrin, isatin, and alloxan color reactions performed on Whatman No. 1 paper.]

		$R_f \times 100$		Sensitivity Limits					
				Ninhydrin		Isatin		Alloxan	
	Amino Acid[a]	Phenol-H_2O, 0.1% NH_3	Butanol-HAc-H_2O	γ	Color	γ	Color	γ	Color
1.	Alanine	64	40	0.2	Pu.	5	L	0.4	P
2.	β-Alanine	72	48	0.4	Pu.	N.R.		0.4	R-P
3.	α-Aminobutyric acid	75	50	0.1	Pu.	0.1	B	0.4	P
4.	γ-Aminobutyric acid	84	54	0.5	Lt. Pu.	0.5	Pu.	0.5	R-P
5.	β-Aminoisobutyric acid	82	58	2.6	Pu.	10	B	0.2	P
6.	Arginine	86	25	0.2	Pu.	2.0	L	1.0	P
7.	Asparagine	50	24	0.7	Gy.-Pu.	N.R.		1.3	R-P
8.	Aspartic acid	24	31	0.3	Pu.	3	B-G	0.7	P
9.	Canavanine	71	22	0.3	Pu.	13	L	0.4	P
10.	Carnosine	88	17	5.7	B	20	B	0.7	P
11.	Citrulline	70	25	0.9	Pu.	3.5	L	0.8	P
12.	Cystathionine	36	15	0.4	Pu.	1.9	B-Gy.	0.2	P
13.	Cysteic acid	15	18	0.6	L-Pu.	0.8	L	0.8	P
14.	Cysteine	39	13	2.7	Lt. T	3.6	B-G	0.8	T-P
15.	Cystine	18	18	0.5	B-Pu.	1.2	B-Gy.[b]	0.8	P-Pu.
16.	3,4-Dihydroxyphenylalanine	38	35	4.9	Br.	0.2	Br.	0.3	Br.
17.	3,5-Diiodotyrosine	65	70[c]	2.0	Gy.	2.0	G	2.2	T-P
18.	Djenkolic acid	47	14	1.3	T	2.5	T	0.8	T
19.	Ethionine	87	65	0.2	Y-Pu.	1.6	B-G	0.2	P
20.	Glutamic acid	35	36	0.3	Pu.	4.0	L	0.8	P
21.	Glutamine	33	35	1.5	Pu.	N.R.		3.0	Lt. P
22.	Glutathione (oxidized)	20	21	0.1	Pu.	5	Gy.-T	4	P
23.	Glutathione (reduced)	17	12	1.5	Pu.	6.2	Pu.	1.4	P
24.	Glycine	50	31	0.2	Pu.	4	Lt. L	0.4	P
25.	Histidine	80	23	0.4	Gy.-Pu.	2.0	B-Gy.	1.0	P
26.	Homocysteine	44	24	0.3	Y-Pu.	1.0	B-G	0.3	P
27.	Homocysteine thiolactone	46	19	15.0	Pu.	3.8	P	0.2	P
28.	Homoserine	64	38	0.04	Pu.	10	P	0.8	P
29.	Hydroxylysine	70	16	0.2	Pu.	0.8	Pu.-Gy.	0.4	P-L
30.	Hydroxyproline	71	33	0.6	Y	1.3	B	N.R.	
31.	Isoleucine	86	73	0.1	Pu.	10	L	0.7	P
32.	Lanthionine	35	14	0.2	Br.	0.4	T	0.2	Br.
33.	Leucine	86	73	0.1	Pu.	10	L	0.7	P
34.	Lysine	86	22	0.1	Pu.	2.0	Br.	0.5	P
35.	Methionine	80	55	0.1	Pu.	0.7	B-G	0.8	P
36.	Methionine sulfone	70	35	0.08	Pu.	4	Gy.	0.4	P
37.	Methionine sulfoxide	82	36	0.08	Pu.	4	B-G	0.4	P
38.	Methylhistidine	92	28	0.04	Gy.-Pu.	2.0	B-G	0.6	P
39.	Norleucine	87	76	0.1	Pu.	2.0	B	0.7	P
40.	Norvaline	83	71	0.1	Pu.	2.0	B	0.6	B
41.	Ornithine	83	19	0.3	Pu.	0.3	Pu.	0.6	P
42.	Phenylalanine	86	66	0.7	Pu.	0.7	B-G	0.8	P
43.	Phenylserine	77	57	0.04	L	10	P	0.6	Y-P
44.	Proline	92	44	0.5	Y	0.2	B	N.R.	
45.	Serine	43	31	0.2	Pu.	3	Lt. L	0.5	P
46.	Taurine	52	23	0.6	Lt. Pu.	N.R.		0.6	P
47.	Thiolhistidine	37	25	0.8	Y-Br.	1.9	B-Gy.	0.4	P
48.	Threonine	55	36	0.1	Pu.	3	Lt. L	0.6	P
49.	Tryptophan	74	47	0.2	Pu.	0.8	B-G	1.0	P
50.	Tyrosine	64	45	0.2	Br.	0.9	B-G	2.0	T
51.	Valine	80	62	0.1	Pu.	10	L	0.6	P

Legend for Table: B = Blue; Br. = Brown; D = Dark; G = Green; Gy. = Gray; L = Lavender; Lt. = Light; P = Pink; Pu. = Purple; R = Red; T = Tan; Y = Yellow; N.R. = No reaction.

[a] The following substances were tested and gave weak or no reactions with all three color reagents: sarcosine (alloxan, faint reaction), betaine, guanidine, and glycocyamine.

[b] This value for cystine is somewhat lower than that previously reported with isatin (*44*).

[c] This material gave rather diffuse bands with this solvent, so that the R_f value is only an approximation.

RESULTS

In agreement with Rao and Giri (*36*) it was found that butanol–acetic acid–water is a most effective solvent for the well-defined separation of amino acids. It was also confirmed that circular paper chromatography gives somewhat higher R_f values with this solvent than are obtained by other techniques (*46*). While there are some differences between the R_f values for this solvent as reported in Table I and those found by Rao and Giri (*36*), the sequence of the various amino acids is virtually the same.

The widely used phenol-water developing solvent gives more diffuse bands than does the butanol–acetic acid–water system (*36*). However, phenol-water is most useful because, in the presence of 0.1% ammonia as an additional phase, it separates into pure bands those commonly occurring amino acids not resolved by the butanol solvent (*34*). The R_f values given in Table I for phenol are in better agreement with those reported (*9*) for the descending technique, than for the circular method (*36*). It has been reported (*9*) that the use of an ammonia atmosphere in the phenol runs results in an improved separation of the basic and acidic amino acids. This would explain the wide divergence between the results for these amino acids reported by Rao and Giri (*36*) and in the present study.

Rao and Giri (*36*) and other investigators (*9, 35*) have tended to minimize the importance of exact R_f values and consider them as a "mere indication of the relative positions of the acids with respect to each other." Admitting that the order of separation of amino acids is of prime importance, it has been demonstrated that the circular chromatographic method, as modified for a closed system, permits one to obtain average R_f values which are reproducible to about ±0.02 unit (*34*). This makes such R_f data useful for identification purposes. Although slight differences in these R_f values may be obtained from one laboratory to another because of differences in paper, reagents, equipment, and temperature, consistent R_f values within the experimental error can be obtained in any one laboratory under controlled experimental conditions.

One might expect to obtain separations of amino acid mixtures whose R_f values differ by ±0.04 unit for a particular solvent. However, this cannot be predicted in advance without experimental trial, because other substances present may exert an effect on the mobility of a compound (*41*). In certain instances it has been found possible to identify amino acids whose R_f values differ by only 0.01 or 0.02 unit, as in the case of lysine, arginine, and histidine (*34*). Identification is based on their distinctive color reactions with isatin and ninhydrin, rather than on the use of a multiplicity of solvents which increase the complexity of the method. Such identification is also obtained in the case of overlapping amino acids where one of the mixtures gives a distinctive color reaction with a differential reagent (*44*).

The limits of sensitivity of the ninhydrin reaction for some amino acids using two-dimensional chromatography has been reported by Dent (*12*), Auclair (*3*), and Berry (*4*). The results obtained in the present study with this reagent, as shown in Table I, compare most closely with those obtained by the latter two authors. In general, the results tend to be slightly lower than those reported by Auclair (*3*). However, in the case of certain amino acids such as arginine, tryptophan, threonine, etc., which are partially destroyed by phenol, either during the developing or drying process (*14, 15*), considerably lower values were obtained. This is due to the fact that sensitivity limits were determined as spot tests on filter paper and not on chromatograms. The useful sensitivity limits on actual chromatograms would probably be about two to three times greater than those reported in Table I. In the case of circular paper chromatography, larger amounts of the amino acids are required for visual detection with increasing R_f values. This in turn is dependent on the solvent system used. It should, perhaps, again be stressed that successful application of the color reactions described here to actual chromatograms requires complete removal of phenol by washing with organic solvents prior to the heating step (*14, 15*). This is most readily performed where small circles, or strips, of filter paper are used for the chromatography.

In general, ninhydrin is the most sensitive of the three amino acid reagents investigated. It gives purple or bluish colors with almost all amino acids except proline and hydroxyproline (yellow); 3,4-dihydroxyphenylalanine, tyrosine, lanthionine, thiolhistidine, cysteine, and djenkolic acid (brown or tan); and 3,5-diiodotyrosine (gray).

Alloxan, whose structure resembles ninhydrin closely, also gives brownish colors with the same acids listed above, except for proline and hydroxyproline, with which it gives no color. Pinkish colors are obtained with practically all the other amino acids tested. Alloxan should prove to be a most useful new color reagent for the detection of small amounts of certain amino acids. Its reactivity with such acids as β-aminoisobutyric acid, carnosine, cysteine, cystathionine, and homocysteine thiolactone is far superior to that obtained with either ninhydrin or isatin. In addition, the colors obtained on paper chromatograms are surprisingly uniform and stable over long periods of time with practically all the commonly occurring amino acids; this is in contrast to the more rapidly fading ninhydrin colors. In general, its sensitivity for most amino acids is only slightly less than is found for ninhydrin, and is much superior to isatin.

Isatin differs from either ninhydrin or alloxan in giving a wider variety of colors with amino acids (Table I). It gives no discernible color reaction with a number of ninhydrin-reacting substances such as asparagine, taurine, glutamine, and β-alanine. In addition, it gives very weak reactions with a number of other amino acids such as glycine, homoserine, glutamic acid, canavanine, phenylserine, valine, leucines, carnosine, serine, threonine, and alanine. These amino acids give colors which do not intensify much with increasing concentration and which tend to fade rather rapidly. Because of these characteristics, isatin is a most useful color reagent in helping to differentiate certain amino acids in mixtures with similar R_f values. Some examples of its use have been cited in previous publications (*34, 44*). Other interesting findings from the present data include: (*a*) the large difference in sensitivity obtained with isatin between the β- and γ-aminobutyric acids with similar R_f values; (*b*) the distinct blue color of norleucine with isatin, while leucine and isoleucine give faint levender colors; (*c*) the use of isatin to distinguish norvaline (blue) from valine (lavender); (*d*) the fact that isatin is equal to or greater in its sensitivity than either ninhydrin or alloxan for the following amino acids: proline, 3,4-dihydroxyphenylalanine, 3,5-diiodotyrosine, α- and γ-aminobutyric acids, ornithine, and phenylalanine. It is, therefore, to be expected that depending on the complexity and content of the amino acid system to be analyzed, one or more of these color reagents can be used to obtain identifications even where complete chromatographic resolution has not been achieved.

DISCUSSION

Although ninhydrin and isatin are among the two most widely used color reactions for amino acids (*9, 34, 48*), the reaction which takes place was originally investigated by Strecker (*47*) for alloxan. Subsequent work has shown that these three substances and others possessing the group $—CO(CH=CH)_nCO—$ react in a fairly general manner with α-amino acids having the structure $RCH(NH_2)COOH$. In each instance, the reactive amino acid is degraded to the corresponding aldehyde having one less carbon atom (*45*), with carbon dioxide and ammonia being liberated during the reaction. Although liberation of both carbon dioxide (*50*) and ammonia (*28*), as well as aldehyde formation (*51*), has been used for the quantitative determination of α-amino acids, this discussion is concerned only with the nature of the colored substances formed in these reactions. Of these three reagents, the mechanism of the reaction of ninhydrin with α-amino acids has been recently studied by Moore and Stein (*31*) and MacFadyen and Fowler (*29*). The purple color (Ruhemann's purple) is attributed to the anion of diketohydrindamine-diketohydrinylidene. Under the usual experimental conditions, such as those employed here, both the molar color yields (*31, 32*) and the spectral curves (*13*) differ within a series of amino acids. However, under controlled conditions of pH and temperature, and in the presence of organic solvents having maximal water content, it is possible to obtain purple color yields which approach 100% of theory (*13, 49*). These conditions do not apply to the reaction of ninhydrin with the imino acids proline and hydroxyproline. With these acids, although carbon dioxide is evolved, deamination does not occur and the acid residues condense directly with the ninhydrin to form the yellow pigments (*49*).

According to Abderhalden (*1*), the pink-colored components formed by the interaction of alloxan with amino acids are of unknown constitution. However, because of the similarities in the structure and reactivities of alloxan and ninhydrin, they are believed to be of a composition similar to Ruhemann's purple (*10*). This viewpoint is supported by the present experimental data in that all amino acids which gave purple colors with ninhydrin gave pink colors with alloxan, and all those which gave other shades with one reagent also did so with the other. There are, however, marked differences in their sensitivities which may be due to steric factors or side reactions.

A mechanism for the Strecker reaction of α-amino acids with isatin, similar to that for ninhydrin and alloxan, has been proposed by Schönberg and others (*45*) in which the colored product formed is isatide. Again, as in the case of ninhydrin, the blue-colored products formed with the imino acids are believed to be of an entirely different structure than the other amino acids—i.e., pyrrole blues (*20*). Because of the somewhat different structure of isatin, as compared to the other two reagents, its reaction with various amino acids would not be expected to parallel their reactions. However, on the basis of such a mechanism, it would still be difficult to explain the wide variety of colors obtained with this reagent which make it most useful in paper chromatography. In addition to the possibility of side reactions and steric factors accounting for some of these color differences, they may represent various oxidative stages of the products formed during the reaction (*24*). This would account for the weak colors formed and the relative ease with which they fade from the paper chromatograms.

Because reagents described are general reagents for the α-amino acid grouping, the authors have preferred the terms "selective" or "differential" reagent to the more commonly used "specific" reagent. This latter term is more applicable to reagents such as the phenol reagent for tyrosine or the Sakaguchi reaction for arginine, although it is also used loosely to apply to reagents such as platinic iodide or phenol-hypochlorite which react with a number of amino acids (*4, 6*). Such specific reagents are readily applied to small sectors from the circular chromatograms to supplement the differential reagents discussed here. While not so convenient, specific reagents are useful in helping to confirm the presence or absence of a particular amino acid in a band containing a mixture.

Many of the sensitivity data reported here for amino acid reactions with isatin and alloxan on paper, and some of them for ninhydrin, are new to the literature. These data should be useful to investigators engaged in paper chromatography of amino acids. They also hold promise for the development of new methods for the quantitative determination of such acids as norleucine, norvaline, the prolines, etc., as well as for peptides and proteins. Work in this direction is at present in progress in this laboratory.

ACKNOWLEDGMENT

The authors wish to acknowledge the aid and encouragement given them by Bruno W. Volk, Director of Laboratories, who permitted this work to be performed under Grant B-285 of the U. S. Public Health Service, and to Lillian Salowitz for the typing and editing of the manuscript. The authors also gratefully acknowledge the gift of a number of amino acids included in this study from Louis B. Rockland, U. S. Department of Agriculture Laboratory, Pasadena, Calif., and from Alfred Deutsch, California Foundation for Biochemical Research, Los Angeles, Calif.

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RECEIVED for review October 25, 1955. Accepted December 27, 1955. Eighth annual meeting-in-miniature, New York Section, ACS, March 1956.

Analysis of Boron Tribromide and Its Addition Compounds

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Several methods of determining the boron and bromine content of boron tribromide and its addition compounds were compared. The sample was hydrolyzed and the organic portion removed by means of an ion exchange resin to yield an effluent containing boric and hydrobromic acids. The inorganic acids were titrated with sodium hydroxide by four different methods: potentiometric, conductometric, visual, and spectrophotometric. Various acid-base indicators were employed. The potentiometric and spectrophotometric methods proved to be equally accurate, but the spectrophotometric titration was faster.

AN EXAMINATION of the literature on the analysis of boron tribromide indicates that relatively poor accuracy is usually obtained in the boron analysis (*3*, *4*, *6*, *7*). Moreover, the analysis of boron tribromide addition compounds is further complicated by the fact that the amount of boron in the addition compound is usually only a small percentage of the total composition. The method most frequently used is hydrolysis of the salt, titration of the total acids (boric and hydrobromic) with standard sodium hydroxide, followed by a determination of the bromine content by titrating with silver nitrate. The boric acid is then obtained by difference.

In the present work it was found that the basicity of the organic molecules used in preparing the addition compounds interfered with the determination of the acids produced upon hydrolysis. The titration values were inaccurate because of salt formation between the organic base and the hydrobromic acid. To overcome this difficulty, the hydrolysis mixture was passed through a cation exchange resin, Amberlite IR-100, hydrogen form. The organic base displaced its equivalent of hydrogen ion and was itself retained on the resin column. Some examples of the organic bases removed by this method are acridine, 5-nitroquinoline, morpholine, pyrrole, piperidine, 2-, 3-, and 4-picoline, isoquinoline, and nitrobenzene.

The effluent containing the hydrobromic and boric acids was collected. The acids were quantitatively recovered in the effluent. A tenfold volume of wash water was found to be adequate to ensure that all the acids produced were transferred from the column to the collecting beaker. This amount of washing resulted in a solution approximately 0.02 to 0.03N in acid.

To evaluate various methods for determining the amount of hydrobromic and boric acid released, standard solutions were prepared and mixed to give an approximately 3.6 to 1 ratio of hydrobromic to boric acid—i.e., a ratio of bromine to boron of 3.6 to 1. These methods are compared in the body of the paper.

REAGENTS

A 0.3N carbonate-free sodium hydroxide solution was prepared and standardized against standard hydrochloric acid. Standard solutions of about 0.015N hydrobromic acid and about 0.005N boric acid were prepared with carbon dioxide-free water.

Methyl red, methylene blue, and phenolphthalein indicators were prepared in the usual way. The mixed indicator (*1*) was prepared by mixing 40 ml. of methyl red with 15 ml. of methylene blue solution.

Table I. Comparative Titrations by Different Methods

(Br/B Ratios Given)

Visual	Spectrophotometric	Potentiometric	Conductometric
Methyl Red and Phenolphthalein			
3.482	3.604	3.604	3.688
3.578	3.632	3.604	3.545
3.594	3.604	3.604	4.000
3.456	3.604	3.604	3.606
	3.606	3.609	
Mixed Indicator and Phenolphthalein			
3.488	3.604		
3.474	3.604		
3.530	3.576		
3.515	3.604		
	3.610		

APPARATUS

A Leeds & Northrup pH meter, Type 7662, with glass and calomel electrodes and a magnetic stirrer for agitation, was used in potentiometric titrations.

A Beckmann Model B spectrophotometer was adapted to use a titration cell similar to that described by Sweetser and Bricker (*8*). A Rehberg microburet was adapted with a delivery tube to permit titrations in the spectrophotometer cell.

An Industrial Instruments conductivity bridge, Model RC 1B, with platinized platinum electrodes and a magnetic stirrer was used in the conductometric titrations.

COMPARATIVE TITRATIONS

Potentiometric Titration. One hundred milliliters of the test solution were titrated potentiometrically with standard sodium hydroxide solution, using a glass electrode and a calomel cell. After the first end point (due to the neutralization of hydrobromic acid), approximately 5 grams of mannitol (*5*) was added and the titration continued until all the boric acid was neutralized. For most of this work and for general use, a 10-ml. microburet graduated in 0.02 ml. was used. However, to follow the instrument readings through the rapidly changing region of the end point, the titrations were taken to within 0.1 ml. of the end point, determined previously with a 10-ml. microburet. A Rehberg microburet was employed to add 0.005-ml. increments of the same strength base. The results are given in Table I.

Visual Titration with Indicators. METHYL RED. Five drops of the methyl red indicator was added to 100 ml. of the test

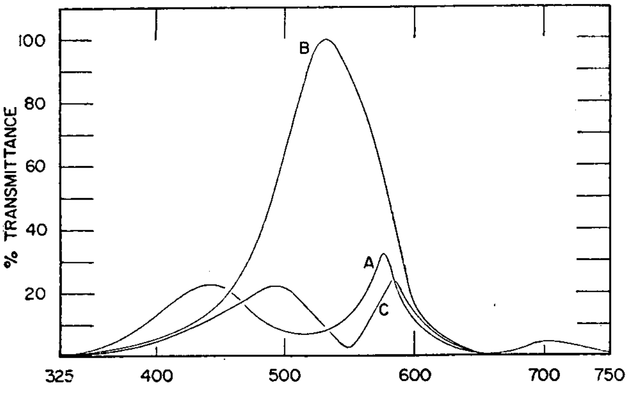

Figure 1. Transmittance curves for mixed indicator

A. Methyl red—methylene blue (acid)
B. Methyl red–methylene blue (basic)
C. Methyl red–methylene blue with phenolphthalein and mannitol (basic)

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solution, which was then titrated with standard sodium hydroxide solution to the yellow end point. Approximately 5 grams of mannitol and 5 drops of phenolphthalein were added and the titration was continued to the pink color of phenolphthalein. Results obtained by this method are included in Table I.

MIXED INDICATOR. The titration with mixed indicator was carried out in the same manner as the methyl red titration, using 5 drops of mixed indicator instead of methyl red. The titration was carried to the gray-green end point, then, after the addition of mannitol and phenolphthalein, to the red-violet color of the mixed indicator. The results are also given in Table I.

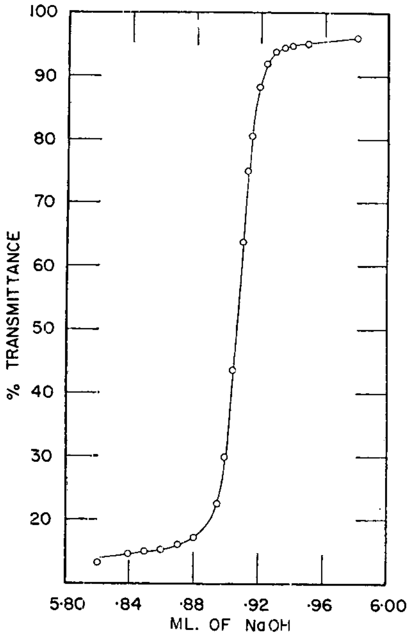

Figure 2. Spectrophotometric titration curve of hydrobromic acid in hydrobromic–boric acid mixture

Methyl red–methylene blue mixed indicator

Spectrophotometric Titration. MIXED INDICATOR. The absorption spectra of the mixed indicators in the test solution were determined (Figure 1, curve *A*), then the hydrobromic acid was titrated with a solution of sodium hydroxide and the absorption spectra were determined again (curve *B*). Mannitol (5 grams) and phenolphthalein (5 drops) were added, the boric acid was titrated, and a final absorption spectrum was determined (curve *C*). From these three spectra the optimum wave length for the titration was determined as 532.5 mμ. At this wave length, in order to keep the readings on the scale at all times during the titration, the slit width was maintained at 0.14 mm.; this may vary with the instrument and should be determined before the titration. When the apparatus was on these settings, the end point occurred at 50% transmittance. The curve obtained in the spectrophotometric titration of hydrobromic acid with sodium hydroxide is shown in Figure 2. Figure 3 shows the titration of boric acid with sodium hydroxide. A summary of the results is given in Table I.

METHYL RED INDICATOR. By following the procedure used for the mixed indicator, the optimum wave length for the titration with methyl red was determined to be 550 mμ. The slit width was 0.17 mm., and 50% transmittance corresponded to the end point. Spectrophotometric titrations of hydrobromic acid and of boric acid with sodium hydroxide were made. The results are summarized in Table I.

Conductance Titration. The platinized platinum electrodes of the conductivity bridge were immersed in 100 ml. of the test solution and the resistance was measured. Increments of 0.5 ml. of standard sodium hydroxide solution were added and the resistance was measured after each addition. Resistance readings were then converted to conductance values, corrected for volume changes, and plotted against milliliters of titrant added. The intersections of the straight lines were taken as end points. The results are given in Table I.

Table II. Analysis of Boron Tribromide and Its Addition Compounds

(Spectrophotometric titrations with mixed indicator and phenolphthalein)

Compound	HBr, Meq.	H_3BO_3, Meq.	Br/B Ratio
Boron tribromide[a]	1.551	0.517	3.000
Addition compounds[b]			
5-Nitroquinoline .	0.962	0.320	3.006
Quinoline	1.715	0.5757	2.979
4-Picoline	0.514	0.172	2.988

[a] Mandell (*6*) found a Br/B ratio of 3.76 for boron tribromide.

[b] Kinney and Mahoney (*4*) obtained a Cl/B ratio of 4.38 for boron trichloride addition compounds.

ANALYSIS OF ADDITION COMPOUNDS

Approximately 3 meq. of the boron tribromide addition compound were weighed out on an analytical balance and dissolved in 50 ml. of carbon dioxide–free distilled water, warming gently when necessary. The solution was then passed slowly through approximately 65 grams of the cationic exchange resin Amberlite IR-100 packed in a Jones reductor tube measuring 50 cm. in length and 2 cm. in diameter. The effluent and wash water were collected in a 500-ml. volumetric flask, and the column was washed until a total volume of 500 ml. was obtained. Each of the titrations was done on 100-ml. aliquots of the effluent. The results of the mixed indicator spectrophotometric titration of boron tribromide and several of its addition compounds are given in Table II. This procedure has also been used by Henry (*2*) in the analysis of boron tribromide addition compounds.

SUMMARY

Of the four methods investigated, the potentiometric and spectrophotometric methods gave consistently good results. Visual titrations gave consistently accurate values for hydrobromic acid but high values for boric acid. Although conductometric titrations did not give values as reproducible as the other instrumental methods, they gave approximately correct values and did not require the use of mannitol.

To check these procedures further, several analysts used the various methods on aliquots of the test solution and the boron-

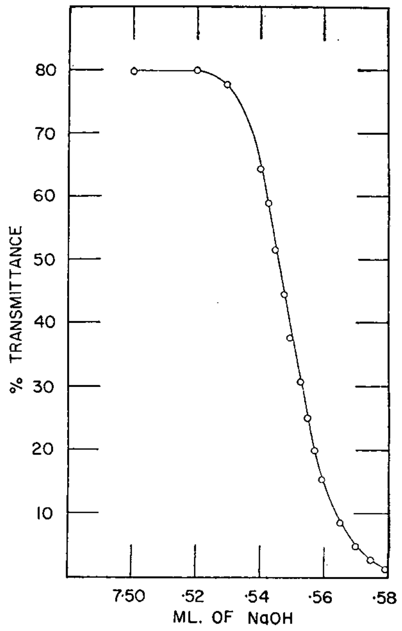

Figure 3. Spectrophotometric titration curve of boric acid in hydrobromic–boric acid mixture

Methyl red–methylene blue mixed indicator with phenolphthalein and mannitol bromide addition compounds. The largest disagreement was apparent in the visual titrations using only methyl red indicator; the mixed indicator visual titration was only slightly better. Data obtained by the various analysts checked very well in both the spectrophotometric and potentiometric titrations. Conductometric titrations were not checked. While potentiometric and spectrophotometric methods are equally satisfactory, the spectrophotometric method appears to be more satisfactory for general use, because it is not necessary to plot the curve to obtain the end point. Once the apparatus has been standardized, the titration can be run to 50% transmittance and the buret reading taken. The needle deflection is also much greater on the spectrophotometer, making it easier to take readings. Spectrophotometric titrations are especially suitable to visual titrations in which the end point is difficult to see.

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RECEIVED for review August 5, 1955. Accepted January 18, 1956. Delaware Valley Regional Meeting, ACS, Philadelphia, Pa., February 16, 1956. Abstracted from a portion of a dissertation presented by William J. Schuele to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of doctor of philosophy.

Available Oxygen in Manganese Dioxide

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Examination of analyses of manganese dioxide for available oxygen indicated very poor agreement among different laboratories. An appraisal was made of the two most important methods used in this analysis—the ferrous sulfate method and the sodium oxalate method. It was discovered that good agreement between the methods could be achieved only with samples of natural origin. It was shown that in the analysis of synthetic manganese oxides, the oxalate method is subject to a positive error because of air oxidation of the oxalate. The methods gave good agreement when a protective atmosphere was provided in the oxalate procedure.

FOR the past several years this laboratory has been concerned with the evaluation of manganese oxides for use as depolarizers in dry cells. As a part of this program, the analysis of the oxides for available oxygen is of importance. The ferrous sulfate method (*5*) was used in specifications defining the minimum available oxygen (*4*). This procedure proved useful in establishing working hypotheses for the correlation of particle morphology and x-ray diffraction powder patterns with manganese to oxygen ratios. Difficulties arose, however, when interlaboratory comparisons were made. Even when the same methods of analysis were used, there were serious discrepancies in results.

A survey of the literature showed this to be a problem of long standing. In 1917, for example, Barnebey (*1*) pointed out that chemists could obtain discrepancies of 5% in the manganese dioxide content of a given sample, while smaller differences were not at all uncommon. This has also been the authors' recent experience.

The ferrous sulfate and oxalate methods seemed to offer most promise as potential referee methods. They are given by the National Bureau of Standards in their certificate for the analysis of manganese dioxide (standard sample No. 25b). Therefore, an investigation was undertaken regarding the general applicability of these methods to manganese oxides with different genetic histories.

EXPERIMENTAL

Ferrous Sulfate Method. A 100-ml. portion of 10% (by volume) sulfuric acid is transferred to a 300-ml. Erlenmeyer flask. Exactly 6 grams of ferrous ammonium sulfate is introduced into the flask and a 0.5-gram sample of the manganese dioxide to be analyzed is added. Carbon dioxide is passed into the flask, the contents are brought to a boil, and the reaction is allowed to proceed until all of the sample is decomposed. Then 5 ml. of sirupy phosphoric acid is added and the excess ferrous ammonium sulfate is titrated with potassium permanganate. At the same time a blank is run with 6.000 grams of the ferrous ammonium sulfate under identical conditions. The available oxygen, calculated as per cent manganese dioxide, is given by the equation

$$\%\ \mathrm{MnO_2} = \frac{4.346\,(B - T)N}{W} \qquad (1)$$

where
W = weight of sample
B = volume of potassium permanganate required for blank
T = sample titer
N = normality of potassium permanganate

Sodium Oxalate Method. A 100-ml. portion of 10% (by volume) sulfuric acid and 1.000 gram of sodium oxalate are added to a 300-ml. Erlenmeyer flask, followed by a 0.5-gram sample of manganese dioxide for analysis. The reaction is allowed to proceed on the steam bath until the sample is decomposed. The excess oxalate is determined by titrating the hot solution with $0.1N$ potassium permanganate. Available oxygen as per cent manganese dioxide is calculated from the equation

$$\%\ \mathrm{MnO_2} = \frac{4.346\,(1/0.067 - TN)}{W} \qquad (2)$$

where the symbols have the same meaning as in Equation 1.

DISCUSSION

Effect of Phase Type and Stoichiometry. For some samples, results obtained with both methods were consistent; for others there was considerable disagreement. In the early stages of the work good agreement had been obtained for a number of naturally occurring pyrolusites. Of the various manganese oxides the pyrolusites (β-manganese dioxide) make the closest approach to stoichiometric manganese dioxide. On the other hand, for other phase types usually having appreciable oxygen deficiencies, considerable discrepancies had been obtained. Phase type (*2*) means a commonly recurring manganese oxide, defined by its x-ray diffraction pattern and by a more or less distinctive morphology. Thus, it seemed that the phenomenon was related in some way to the manganese-oxygen ratio and/or the phase type. In order to test the dependence of analytical inconsistencies on stoichiometry and phase type, two materials, Samples *D* and *O*, were prepared. Sample *D* is a synthetic β-manganese dioxide.

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Table I. Analyses of Synthetic and Naturally Occurring Manganese Oxides

1	2	3	4	5	6
	% Manganese Dioxide				
Sample	$Na_2C_2O_4$, NBS	$Na_2C_2O_4$, + CO_2	$FeSO_4$, NBS	Av. Diff., Col. 2 & 4	Av. Diff., Col. 3 & 4
			Synthetics		
B	78.08	75.96	74.37	3.80	1.11
	78.91	75.65	75.00		
D	99.34	97.59	97.30	2.00	0.30
	99.34	97.76	97.35		
I	90.02	89.69	88.94	1.15	0.73
	89.63	89.18	88.33		
	89.94		88.87		
J	89.71	88.56	88.24	1.41	0.11
	89.35	87.90	88.00		
O	82.29	78.49	77.68	4.17	0.70
	81.83	78.68	78.10		
N[a]	102.2	99.60	99.37	2.52	0.26
	101.6	99.67	99.38		
			Naturals		
C	93.45	93.11	92.75	0.99	0.42
	93.75	92.95	92.47		
E	91.14		90.59	0.45	
	90.91		90.55		
F[b]	90.43		90.30	0.06	
	90.59		90.56		
	90.67		90.57		
H	85.38		85.13	0.02	
	84.92		85.13		
L	80.64		80.08	0.65	
	80.29		79.56		

[a] J. T. Baker manganese dioxide, Lot No. 8271.
[b] NBS standard sample of manganese dioxide, 25b.

It was prepared by thermal decomposition of manganous nitrate. Analytical discrepancies were again obtained. This was confirmed by analysis of Sample *N*, a special J. T. Baker reagent, which became available subsequently. The second preparation, Sample *O*, was the product obtained from the Volhard reaction (*5*) used in the determination of total manganese. This method offered a convenient means of obtaining a nonpyrolusitic MnO_n, where n is almost 2 and can be computed independently from a known empirical factor (*3*). Once again the methods gave inconsistent results (Table I). Thus, the effect could be demonstrated by controlling the phase type or n-value independently.

Naturals *vs.* Synthetics. Typical results are given in columns 2 and 4 of Table I. For the oxides from natural sources, there is reasonably good agreement, except for a slight tendency of the oxalate results to be high with respect to the ferrous sulfate results. On the other hand, for the synthetic materials the oxalate results were uniformly too high, the differences varying from about 1 to 4%. It is noteworthy that the extent of the difference is reproducible for any given sample but varies from one sample to another.

Extraneous Loss of Oxalate. In the oxalate method high results would be caused by a disappearance of oxalate. Two alternative explanations were available. The first possibility was decomposition of oxalate; the second, that it was subject to air oxidation. Because manganous ion may promote the decomposition of oxalate, it was decided first to check this possibility experimentally. This reaction proceeds according to the equation

$$H_2C_2O_4 \xrightarrow[90^\circ\text{ C.}]{Mn^{++}} H_2O + CO + CO_2 \qquad (3)$$

On the other hand, the reaction with manganese dioxide is given by

$$MnO_2 + H_2SO_4 + H_2C_2O_4 \rightarrow MnSO_4 + 2CO_2 + 2H_2O \qquad (4)$$

Carbon monoxide is a reaction product only if there is decomposition of the oxalic acid. Accordingly, an apparatus was set up to permit quantitative measurement of any carbon monoxide which might be evolved during the reaction. In several runs, no carbon monoxide could be detected. Furthermore, when a carbon monoxide generator, consisting of a hot solution of sodium oxalate in concentrated sulfuric acid, was put in series with the reaction vessel, the theoretical amount of carbon monoxide was recovered. The conclusion was reached, therefore, that no catalytically promoted decomposition of oxalate occurred. This agrees with Barnebey's findings (*1*).

Only the second alternative remained to explain the disappearance of an excessive amount of oxalate—namely, by air oxidation. If an air oxidation did occur one would expect the effect to be time-dependent. This, in fact, was found to be the case. The oxalate results on the synthetics were very sensitive to digestion time. Results for the natural materials, on the other hand, were insensitive to variations in digestion time. For a given sample the largest analytical discrepancies were obtained for longest digestion periods. For this reason the oxalate data given in Table I are to be regarded as illustrative only, because digestion periods of 4 hours were used in all cases. Various values intermediate between the ferrous sulfate and the oxalate results may be obtained for shorter oxalate digestion periods. Some samples were in solution long before they were removed from the steam bath. Therefore, the continued disappearance of oxalate must be accounted for, at least in part, by effects of soluble reaction products. It may be inferred that the time needed to dissolve a sample may influence the extent of the positive error. No quantitative data are available on this point. It is noteworthy that the pyrolusites which generally require longer digestion times do not show the largest difference.

Use of Protective Atmosphere. Each of the reaction vessels was fitted with an inlet tube for carbon dioxide (or nitrogen) and an outlet tube. A flow of gas was maintained in the reaction vessel during the entire reaction and up to the time of titration. Some typical results are given in column 3 of Table I. The use of carbon dioxide brings the methods into reasonable agreement, although there is still a residual positive difference in favor of the oxalate method.

CONCLUSIONS

The standard oxalate procedure is subject to a positive error because of air oxidation of oxalate. The extent of oxidation is variable; it depends on sample origin, and is influenced by iron content. For best results, therefore, the sodium oxalate method should be revised to provide an inert atmosphere before it is generally applicable for the determination of available oxygen in manganese dioxide.

No evidence was obtained for the decomposition of oxalate under conditions of the reaction.

ACKNOWLEDGMENT

M. S. Fink of these laboratories set up the train for the detection of carbon monoxide and prepared the Volhard product. Thanks are due also to other members of the staff who supplied supporting data for this work.

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Received for review June 4, 1955. Accepted December 28, 1955.

Analytical Method for Thujaplicins

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To facilitate investigations of the chemistry and variations in the natural durability of western red cedar, a rapid method for the determination of the substituted tropolones (the fungicidal thujaplicins) was developed. The thujaplicins in a hexane-chloroform solvent are treated with aqueous ferric acetate solution to yield their red ferric chelates, which are then estimated colorimetrically in the organic layer. For analysis of cedar wood, the thujaplicins are extracted from the finely ground sample with hot water and then extracted into hexane. This procedure excludes an interfering phenol which is also extracted from the wood by the hot water.

THE α-, β-, and γ-thujaplicins (2-hydroxy-3-, 4-, and 5-isopropyl-2,4,6-cycloheptatrien-1-one) are potent fungicides occurring in the heartwood of western red cedar (*Thuja plicata* D. Donn.) (*1, 6*), eastern white cedar (*Thuja occidentalis* L.) (*7*), hiba (*Thujopsis dolabrata* Sieb. et Zucc.) (*10*), and Formosan hinoki (*Chamaecyparis taiwanensis* Masmune et Suzuki) (*10*).

These extractives are believed to be mainly responsible for the high natural durability of western red cedar (*11*). In investigations of the acetone extractive in the species (*2*), and also of the distribution of the durability factors (*8*), the need for a simple rapid method of determining these substituted tropolones in solutions and in wood substance became evident. A simple accurate method is described which has been used routinely to analyze a large number of small wood samples (*8*).

EQUIPMENT AND REAGENTS

The following are needed:
Fisher Electrophotometer with No. 425 blue filter.
Wiley mill, intermediate model.
Burrell wrist-action shaker.
n-Hexane, boiling point 65–67° C.
Chloroform, ACS reagent grade.
Ferric acetate solution. Dissolve equimolar quantities of sodium acetate and ferric chloride in sufficient distilled water to make a final solution 1% in iron. This solution has a pH of 4.3.

PROCEDURE

Sufficient wood material is ground in the Wiley mill to pass a 40-mesh screen to provide duplicate 2-gram samples for hot water extraction and duplicate 1-gram samples for moisture content determination.

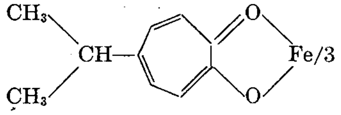
The 2-gram samples are leached for 1 hour with 100 ml. of distilled water in a 250-ml. Erlenmeyer flask, which is placed in a boiling water bath and fitted with an air condenser. The hot liquid extract is carefully decanted through a tared Alundum or fritted-glass crucible. The procedure is repeated, using 50 ml. of distilled water for 1 hour followed by 25 ml. of distilled water for 1/2 hour. The sample is then transferred to the crucible and washed with a maximum of 25 ml. of hot distilled water. The crucible may be dried and weighed for a hot water solubility determination, if required.

When the combined water extracts are cool, they are made up to 200 ml. with distilled water. A 5-ml. aliquot of this solution is extracted twice with 5-ml. portions of *n*-hexane in a small separatory funnel, shaking for 1 minute in the Burrell shaker for each extraction. To the 10-ml. hexane extracts are added 10 ml. of chloroform and 5 ml. of ferric acetate solution. The samples are shaken for 5 minutes in a separatory funnel on the shaker, then the organic layer is filtered into a 25-ml. graduate. The aqueous layer is washed with additional chloroform, and the filtrate is made up to a final volume of 25 ml. Absorbance is measured in the Electrophotometer, using a No. 425 blue filter and a similar hexane-chloroform (2 to 3) solvent as a blank. From a previously prepared calibration plot (Figure 1), relating absorbance to thujaplicin content, the thujaplicin content on a moisture-free wood basis is calculated.

If the thujaplicin material is crystalline or is an oily mixture of isomers, a *n*-hexane solution of approximately 5 mg. per 100 ml. is prepared and a 10-ml. aliquot is used as described above

DEVELOPMENT

Tropolone derivatives exhibit characteristic color reactions with several heavy metal cations due to the formation of stable chelates which are soluble in organic solvents. Thus, the thujaplicins react with copper acetate or cuprammonium solutions to give a neutral green chelate which is very soluble in chloroform and insoluble in water (*3*). With the appropriate amount of ferric iron, a red chelate soluble in organic solvents is formed. Both Nozoe (*9, 10*) and Cook and others (*3*) noted that, with an excess of ferric ion or in the presence of acid, the red chelate which forms at first is converted to a green water-soluble complex. They also suggested that the red ferric chelate might be useful analytically because of the intense color of very dilute chloroform solutions. The formula for the ferric chelate of γ-thujaplicin is



In early work in this laboratory it was found possible to determine the thujaplicin content of chloroform solutions accurately by measuring the green color developed on the addition of excess freshly prepared ethyl ether solutions of ferric chloride (*4*). This method was suitable in the absence of phenols and accurate if water was carefully excluded, because the green complex is very soluble in water and relatively insoluble in organic solvents. In working with western red cedar a method was required for the routine analysis of steam distillates and wood extracts known to contain chloroform-soluble phenolic substances which also give green colors with ferric ion. Preliminary experiments had established that other nonphenolic extractives soluble in organic solvents—i.e., thujic acid and its methyl ester—gave no color reaction with ferric iron. Therefore, the utility of the red ferric chelate was investigated.

A method of carefully controlling the iron addition so that none of the red chelate formed would be converted to the green compound was a primary consideration. Accordingly, a number of ferric complexes of lesser stability, such as the lactate, gluconate, citrate, and acetate, were investigated as the medium for adding the iron. By shaking chloroform solutions of γ-thujaplicin with aqueous solutions of these various complexes it was found that maximum red color response in the organic layer and absence of any green color was obtained using ferric acetate solutions. Blank tests showed that the reddish brown ferric acetate solution did not impart any color to the organic layer.

Both β- and γ-thujaplicin had been isolated from western red cedar, and the possibility that relatively small quantities of the α-isomer were present had not been disproved; therefore, it was necessary to compare the color response of the three isomers and that of the mixture of isomers occurring naturally in western red cedar. Pure samples of the β- and γ-isomers were available from previous work on western red cedar (*2*). A sample of the α-isomer was isolated from eastern white cedar (*Thuja occidentalis* L.) by the method of Gripenberg (*7*).

Aliquots of standard solutions (0.5 gram per liter) of the three isomers in hexane-chloroform solvent were treated with ferric acetate solution by the method described, and the results were plotted (Figure 1). The values for α- and β-isomers almost coincided, with the β-isomer giving a slightly greater response. Both were approximately 20% lower than the values for the γ-isomer.

The plot for γ-thujaplicin was a straight line over the range tested, but the plots for the α- and β-isomers fell off slightly at the higher concentrations. The same divergence was obtained by varying the concentration of solutions of the pure ferric chelates of these two isomers, indicating that the divergence was due to deviation from Beer's law, and not a characteristic of the chelate formation reaction.

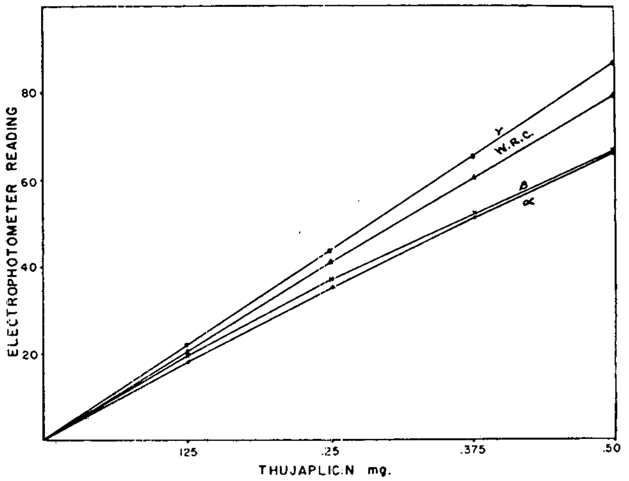


Figure 1. Calibration curves for red ferric chelate of α-, β-, γ-, and western red cedar mixed thujaplicins

The plot obtained using the thujaplicin mixture of isomers isolated from western red cedar is also given in Figure 1. Two separate preparations from different samples of local mill-run western red cedar sawdust, using methods described previously (*2*), gave identical curves (W.R.C.). Because the three isomers have similar stability under the conditions of isolation, the plot obtained should represent closely the mixture of thujaplicin isomers occurring naturally in British Columbia red cedar. Therefore, it was adopted as the calibration curve for analysis of samples grown in this region.

The effect of variation of the pH of the ferric acetate solution on the color formation in the chloroform layer was examined for the β- and γ-isomers. At pH values of 4.3 (normal for ferric acetate solutions) and 4.9, the response for each isomer appeared to be constant. However, at pH 4.9, great difficulty was encountered because of the formation of stable emulsions during the shaking stage. At pH values below 4.3 the color response of the γ-isomer increased, a 12% gain being obtained by reducing the pH to 3.95. However, the value for the β-isomer remained practically constant. Thus, use of a pH below 4.3 increases the divergence between the color response of the two isomers. This would in turn increase any error in the analysis of wood samples caused by the ratio of the thujaplicin isomers being different from that used in obtaining the calibration curve (W.R.C., Figure 1).

A solution of a 1 to 1 mixture of the β- and γ-isomers was tested by the analytical method and gave a ferric chelate color response exactly midway between those obtained for equivalent solutions of the pure β- and γ-isomers, respectively. Therefore, the color developed by a known quantity of a mixture of these two isomers can be used with the aid of the graphs of Figure 1 to calculate the ratio of the isomers occurring in the mixture. If it is assumed that the α-isomer is not present in western red cedar, then the position of the curve for the thujaplicin mixture isolated from wood (W.R.C., Figure 1) relative to those of the β- and γ-isomers indicates that the amount of γ-isomer is approximately 60%. In the event that the α-isomer is found to be present in Canadian-grown red cedar, this rough determination of the content still holds because the color response of the α- and β-isomer is almost identical.

For the direct analysis of wood samples, an efficient method of obtaining the thujaplicin content in solution was required. Steam distillation of cedar wood removes the thujaplicins, which are readily analyzed in colorless steam distillates by the colorimetric method. However, to ensure complete removal of all the thujaplicin, a lengthy distillation at atmospheric pressure is required; therefore, a method of solvent extraction was sought.

It was known from the work of Erdtman and Gripenberg (*5*) that, although diethyl ether is a good solvent for the thujaplicins, it is a poor medium for directly extracting them from the wood, because a large portion of the content is apparently retained in the wood by the ether-insoluble "membrane substances." Hot water extraction appeared to be an attractive possibility in that, by determining the weight of the residual wood meal, there could be simultaneous determination of hot water solubility. Furthermore, the aqueous extract would contain the water-soluble polyphenols, which are derivatives of pyrocatechol (*2*) that had been found to contribute, along with the thujaplicins, to the natural preservative content of western red cedar (*12*).

Table I. Efficiency of Extraction of Thujaplicin from Western Red Cedar Using Hot Water

(2-gram sample)

Stage	Vol. Water, Ml.	Time, Hour	Mg. of Thujaplicin Extracted	% of Total
1	100	1	9.25	78.8
2	50	1	1.72	14.6
3	25	1/2	0.42	3.64
4	25 (wash water)		0.11	0.93
5	25	1	0.15	1.27
6	25	1	0.09	0.76
		Totals	11.74	100.00

If an analytical method for the polyphenols could be devised, aqueous extraction would allow simultaneous determination of hot water solubility, thujaplicin content, and water-soluble polyphenol content.

The efficiency of a hot water extraction of thujaplicin from a sawdust sample was examined and the results are given in Table I.

The first four extractions accounted for approximately 98% of the total material, while the sixth extraction removed only a negligible amount. Therefore, the four-stage extraction was adopted as satisfactory for the analytical method.

Originally it was thought that aqueous extracts of cedar might be analyzed by simply adding ferric acetate solution, extracting the red chelate with chloroform, and measuring its concentration with the colorimeter. However, the mixture of pyrocatechol-type polyphenols contained in the hot water extract of western red cedar (*2*) forms an immediate brown precipitate when ferric acetate solutions are added. This results in emulsions if a subsequent chloroform extraction is attempted. Therefore, it was necessary to extract the aqueous extracts first with a solvent which would completely remove the thujaplicins but only small amounts of the phenols. Chloroform was used initially, but an unexpected difficulty developed. Small quantities of an unknown phenol were found to be extractable with chloroform from the aqueous extracts of cedar. This phenol gave a red color

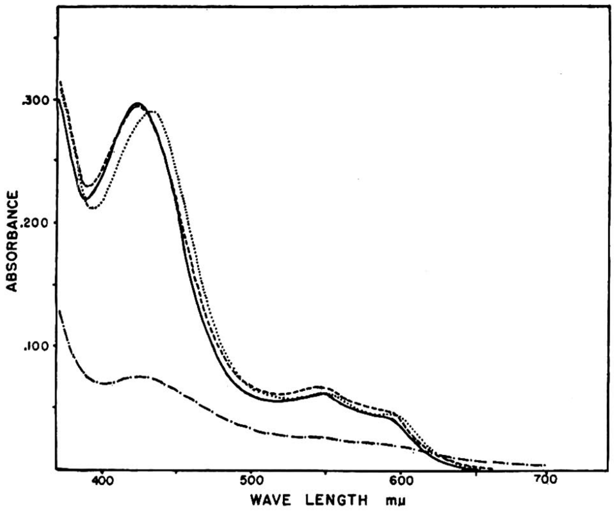


Figure 2. Absorption Spectra

.... γ-Thujaplicin–ferric iron complex
—— Purified western red cedar mixed thujaplicin–ferric iron complex
------ Western red cedar thujaplicin–ferric iron complex by analytical method
·–·–· Interfering color produced by western red cedar polyphenol–ferric iron complex

with ferric acetate in the chloroform layer similar to that of the ferric thujaplicin chelate, and gave results approximately 20% high. Fortunately, experiments with other solvents for the polyphenols showed that the interfering substance was not extractable from aqueous solutions with *n*-hexane which did, however, remove all the thujaplicins. Other compounds extracted by hexane—thujic acid and its methyl ester—do not give any color reaction with ferric ion.

Although hexane is a good solvent for the thujaplicins, it is a poor solvent for the ferric chelates, with the γ-isomer being insoluble and the α- and β-isomers being only slightly soluble. Therefore, to avoid precipitation of the chelates, it was necessary to dilute the hexane solutions with chloroform prior to the treatment with ferric acetate solution. A 3 to 2 dilution by volume of *n*-hexane with chloroform was found satisfactory. This mixed solvent was used in the preparation of the calibration curves and in all analytical work.

The absorption spectra in the visible region for the ferric chelates of the three thujaplicin isomers as determined with a Beckman DU spectrophotometer are very similar, having three maxima. These maxima, as shown in Table II, occur approximately 13 mμ higher for the γ-isomer than for the β-isomer, with those of the α-isomer being approximately midway between the other two. The corresponding spectra for the mixture of thujaplicin isomers isolated from cedar wood, for the chelate color obtained in the analysis of a wood sample, and for the interfering phenol eliminated by the use of *n*-hexane are shown with the spectra of the γ-isomer in Figure 2.

Table II. Maxima in Visible Absorption Spectra of Thujaplicin-Ferric Chelates

Isomer	Maxima, Mμ		
α	426	544	588
β	418	540	584
γ	431	551	595

Again assuming that the α-isomer is absent or present in only minor quantities, the position of the maximum in the 425-mμ region for the ferric chelates of thujaplicins of cedar relative to those of the pure β- and γ-isomers is a rough measure of the relative occurrence of these isomers. Several determinations were made on the color of the chelate from the thujaplicin mixture isolated from different samples of mill-run cedar sawdust. The absorption maximum for these experiments fell at 425 to 426 mμ, indicating that the γ-isomer represented approximately 58% of the mixture. This result is in agreement with the figure of 60% derived previously from the relative intensities of the colors measured.

ACCURACY

The accuracy of the method on cedar heartwood was checked by quantitatively isolating all the thujaplicins as the pure copper salts from a large sample of the wood (50 grams). The yield calculated from the analysis was 0.542 gram, which was 98% of the yield actually obtained (0.55 gram). This result was in line with the 98% efficiency of the extraction phase of the analytical method.

Table III. Analysis of Western Red Cedar Heartwood

Test No.	Thujaplicin, %	Deviation from Av., %
1	0.616	0.65
2	0.611	1.47
3	0.608	1.97
4	0.629	1.43
5	0.628	1.27
6	0.627	1.12
7	0.621	0.16
8	0.621	0.16
9	0.615	0.84
10	0.625	0.80
	Std. dev.	0.0073

Table IV. Analysis of Hot Water Extract of Western Red Cedar Heartwood

Test No.	Thujaplicin, %	Deviation from Av., %
1	0.620	1.61
2	0.610	0.00
3	0.605	0.83
4	0.608	0.33
5	0.620	1.61
6	0.610	0.00
7	0.600	1.61
8	0.610	0.00
	Std. dev.	0.0068

For routine analyses, Figure 1 shows that, if the naturally occurring thujaplicin isomers are in a ratio different from that in the material used to derive the calibration plot (W.R.C.), an error would be introduced. Because it is possible that this ratio would vary somewhat with forest growth conditions, high accuracy would require a simultaneous determination of the ratio, which is impractical for routine work. Moreover, in extensive use of the routine method on wood samples originating from different parts of trees of varying ages from several regions of British Columbia (*8*), no evidence of any wide variation of the ratio was obtained. The same calibration plot was obtained with the thujaplicin mixture from two different samples. Also, the position of the absorption maximum in the 425-mμ region in the spectra of the ferric chelates of several other samples underwent no noticeable shift.

With practice and the use of machine shaking in the extraction stages the precision was very good. The results of ten separate determinations on one sample of western red cedar heartwood sawdust are given in Table III, and those of eight determinations on one sample of aqueous extract in Table IV. Comparison of these results shows that the precision of the aqueous extraction portion of the procedure is very good.

It was noted during the course of this work that actinic light degrades dilute chloroform solutions of the ferric chelates and the thujaplicins rapidly—up to 40% loss in 8 hours of direct sunlight was observed. The chelates were more stable than the free thujaplicins. Therefore, in preparing standards and calibration plots, all solutions were freshly prepared and carefully protected from light during storage.

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RECEIVED for review September 2, 1955. Accepted January 14, 1956.

Semimicrodetermination of Fluorine in Organic Fluoro Compounds

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A simple semimicromethod for the determination of fluorine in organic fluoro compounds is described. The sample is ignited in a modified Parr sodium peroxide bomb and the solution of the melt is percolated through an acidic cation exchanger. The neutralized percolate is titrated with thorium nitrate, using sodium alizarin sulfonate as indicator. In fluoro compounds which also contain chlorine or bromine, the various halogens can be determined simultaneously.

A NUMBER of methods have been proposed for the transformation of organic fluorine into titratable fluoride ions, including: (*a*) oxidation with gaseous oxygen (*7, 14, 25, 33, 35*) or sodium peroxide in a nickel bomb (*13, 24, 27*), with addition of benzoic acid or sucrose as combustion aids and, sometimes, of potassium nitrate or perchlorate as accelerator; (*b*) reduction by the hydrogen flame (*11, 36*) or alkali metal (*16, 17, 20, 23, 30, 34*); and (*c*) fusion with calcium oxide (*1, 21, 29*) or sodium carbonate (*9*). Another method involves the reaction of hydrogen fluoride, formed in the destruction of the organic fluoro compound, with silica (*10, 12, 18*). Occasionally the organically bound fluorine is liberated hydrolytically by aqueous ammonia solution (*26, 30*).

The present investigation, which has already been briefly noted (*15*), has shown that the combustion with sodium peroxide is the most convenient and reliable method; however, the 2.5-ml. Parr microbomb which is ignited by flame did not give reliable results, and the 22-ml. electric ignition bomb had too large a capacity. An electrical ignition bomb of 8-ml. capacity was, therefore, constructed and used successfully.

The fluoride ion formed in the combustion is contaminated with a relatively large quantity of sodium hydroxide and other sodium salts which interfere with the subsequent fluoride determination. Gravimetric determinations [calcium fluoride or lead chlorofluoride (*37*)] failed at the fluoride concentrations studied because precipitation was not quantitative. The usual distillation method (*38*), even after a number of improvements, tended to give low results, especially when the fluorine content was small. It therefore proved necessary to eliminate the sodium ion from the fluoride solution. This was achieved by means of the acid form of a cation exchanger. In the percolate, which contained, in addition to hydrofluoric acid, small quantities of carbonic and hydrochloric acids (when potassium perchlorate was used as combustion accelerator), the fluoride ion can be determined by titration with thorium nitrate, using sodium alizarin sulfonate as indicator. This method (*8*) has been improved by some minor modifications.

EXPERIMENTAL

Reagents and Solutions. Standard sodium fluoride solution (0.1 mg. of fluoride ion per ml.) was prepared by dissolving 0.221 gram of sodium fluoride (Baker analyzed reagent recrystallized once from distilled water and dried) in distilled water, adding 1 ml. of 0.1*N* sodium hydroxide solution, and making up to 1 liter.

Thorium nitrate solution. An approximately 0.018*N* solution was prepared by dissolving 5 grams of thorium nitrate (tetrahydrate, Baker analyzed reagent) in 2 liters of distilled water. No change was observed in the sodium fluoride and the thorium nitrate solutions, which had stood during a period of at least 3 months at room temperature.

Sodium alizarin sulfonate reagent. About 50 mg. of the reagent (British Drug Houses) was dissolved in 100 ml. of distilled water.

Buffer solution, pH 3.55. About 8.2 grams of sodium acetate (AnalaR) was dissolved in 100 ml. of distilled water (approximately 1*M*). To this solution, a 1*M* acetic acid (AnalaR) solution was added to pH 3.55 on a Beckman Model G pH meter.

Figure 1. Peroxide bomb, assembled

Sodium peroxide. Parr calorific sodium peroxide and the Baker analyzed reagent were used with equal results.

Hydrochloric acid (AnalaR), approximately 0.1*N*.

Ethyl alcohol, redistilled once.

Sucrose, AnalaR.

Potassium perchlorate, Parr Co.

Ion exchanger. In the present investigation, Amberlite IR-112 (Rohm & Haas, Philadelphia) was used. Rohm & Haas have discontinued the production of this specific ion exchange resin, and have substituted for it Amberlite EX-100. Any acidic ion exchange resin can be used in the method described.

Apparatus. An 8-ml. semimicro electric ignition bomb was constructed (Figures 1, 2, and 3). The cups were made of stainless steel, the other parts of nickel-electroplated brass. A length of 3.5 cm. of fuse wire (Parr Co.) was used, and a resistance of 1 ohm was incorporated into the ignition circuit. The water bath and ignition unit used were those of the original Parr instrument (*28*).

Procedure. PREPARATION OF CALIBRATION CURVE. With an automatic microburet of 5-ml. capacity, 1, 2, 3, 4, and 5 ml. of the standard sodium fluoride solution were measured into five Erlenmeyer flasks of 50-ml. capacity. Water was added to make a total of 10 ml. of solution. The reagents were then added to each flask in the following order: 0.2 ml. of indicator solution; hydrochloric acid (approximately 0.1*N*) drop by drop, until the color turned from red to yellow; 10 ml. of ethyl alcohol; and finally, 2 ml. of the buffer solution. The solution was titrated with the thorium nitrate solution, using an automatic 5-ml. microburet. The end point was the change of color from orange to red.

Each determination was repeated five times (Table I), and the mean values were plotted. The calibration curve was checked for each new solution of thorium nitrate and, if necessary, corrected.

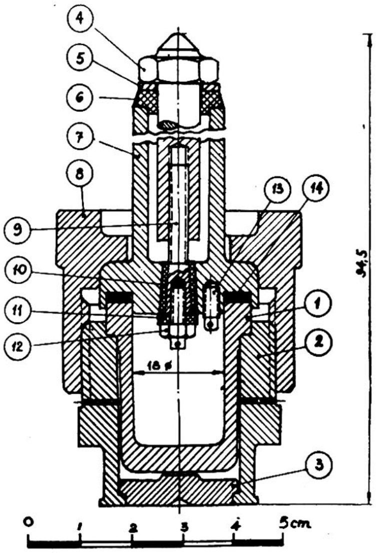

Figure 2. Peroxide bomb, cross section

Preparation of the Ion Exchange Column. Pretreatment of Amberlite IR-112. In a 600-ml. beaker, about 115 grams of Amberlite IR-112 was washed several times with distilled water, 5% sulfuric acid, 8 to 10% sodium hydroxide solution, and finally with about 1 liter of 5% sulfuric acid. Each operation was continued until the supernatant appeared to be colorless. Then the Amberlite was washed thoroughly with distilled water until no more sulfate ions could be detected in the washings.

Preparation of the Column. The lower part of a borosilicate glass tube of 30-mm. inner diameter and approximately 500-mm. length was constricted over a length of 100 mm. to an internal diameter of 10 mm., ending in a fine tip. The inside of the tube (total length 600 mm.) was cleaned thoroughly with chromic acid–sulfuric acid mixture, then coated with paraffin wax. The tip was also coated on the outside to a height of about 3 cm. to prevent any creeping of the percolated solution. The tube was then filled with the pretreated ion exchange resin as uniformly as possible to prevent channeling, leaving a free space of about 5-cm. length at the top of the column.

Table I. Calibration of Thorium Nitrate Solution

F^-, Mg.	Thorium Nitrate Solution, Ml.					Mean Value	Error, %
0.1	0.32	0.32	0.32	0.32	0.31	0.318 ± 0.002	0.6
0.2	0.64	0.63	0.64	0.65	0.65	0.642 ± 0.004	0.5
0.3	0.95	0.96	0.96	0.96	0.96	0.958 ± 0.002	0.2
0.4	1.24	1.23	1.23	1.23	1.24	1.234 ± 0.009	0.7
0.5	1.52	1.52	1.52	1.52	1.53	1.522 ± 0.002	0.1

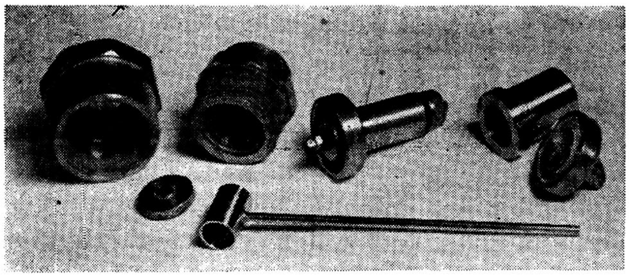

Figure 3. Peroxide bomb, disconnected parts

Regeneration. After each analysis the resin was regenerated by passing 1 liter of a 5% sulfuric acid solution through the column at a rate of approximately 2 ml. per minute. This was followed with distilled water until no sulfate ions could be detected in the percolate. The regeneration and washing of the column were carried out with the help of a simple device shown in Figure 4. A bottle of approximately 1-liter capacity was filled with the liquid, then fitted with a stopper having a short glass tube of 10-mm. diameter and about 100-mm. length through it. The bottle was put on a ring support upside down above the column. The glass tube protruding from the stopper entered the empty space at the upper part of the column. This device eliminates any overflow and works unattended.

Analytical Method. A sample of 20 to 60 mg. of the dried and powdered organic fluoro compound is weighed into the stainless steel cup of the combustion bomb. The weight of the sample is chosen so that the final concentration of fluoride ion is 0.1 to 0.3 mg. per 10 ml. of solution.

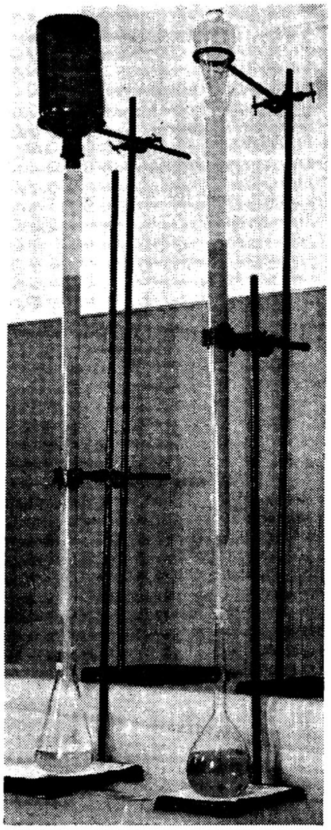

Figure 4. Ion exchange columns

***Left*, assembled for regeneration**
***Right*, assembled for analysis**

The sample is mixed with dry powdered sucrose (analytical reagent) to a total weight of 200 mg. About 4 grams of sodium peroxide is added with a stainless steel spoon (internal diameter, $^{33}/_{64}$ inch, inside depth $^{27}/_{32}$ inch), and the contents are mixed thoroughly with a glass rod. Sometimes up to 200 mg. of potassium perchlorate was added to the mixture as accelerator. A few crystals of sucrose are sprinkled on the surface, and the head, to which a 3.5-cm. fuse wire is attached, is screwed on. Ignition is carried out as usual (*28*).

In case of liquid samples, the compound is weighed into a gelatin capsule which is then buried in the mixture of sucrose and sodium peroxide, giving a total weight of 200 mg. of combustible matter including the capsule.

After ignition and cooling, the bomb is removed from the water bath and the exterior of the stainless steel cup is washed with distilled water. The cup is then transferred to a 150-ml. beaker and covered with 50 ml. of distilled water. The beaker is covered with a watchglass and heated on a steam bath; digestion is continued until the effervescence subsides. The cup is removed and rinsed with distilled water, and the washings are combined with the solution, which is then filtered and washed into a separatory funnel. The solution is passed through the Amberlite column at a rate of approximately 35 drops per minute, so as to prevent any accumulation of liquid on top of the column. The percolate is collected in a 250-ml. volumetric flask, containing about 10 ml. of 0.2N sodium hydroxide solution and 2 drops of phenolphthalein indicator solution. The percolate must drop straight into the alkaline solution without coming into contact with the neck of the flask prior to neutralization. The solution is washed down with distilled water, filled to the mark, and mixed. During this time, the solution in the volumetric flask must remain alkaline to phenolphthalein. In case the solution becomes acid, sodium hydroxide must be added immediately to prevent any loss of fluorine by interaction of the hydrofluoric acid with the glass.

Aliquots of 10 ml. of solution were titrated as for the calibration curve. The fluorine content was found from the volume of thorium nitrate solution, with the help of the calibration curve.

RESULTS AND DISCUSSION

The results of some determinations are given in Table II.

The mean error in these determinations is about 0.6%, which is the approximate error of the titration (see Table I).

Other halogens, sulfur, nitro, and amino groups do not interfere with the determination, and aliphatic and aromatic fluorine can be determined with equal accuracy.

Table II. Examples of Fluorine Determinations

Compound	Formula	Fluoride Ion, %		Error, %	Literature Cited
		Calcd.	Found[a]		
Fluoroacetamide	C_2H_4ONF	24.65	24.86	0.8	(*2*)
4-Fluorobiphenyl	$C_{12}H_9F$	11.03	11.11	0.7	(*32*)
ω-Fluoro-*m*-nitroacetophenone	$C_8H_6O_3NF$	10.37	10.43	0.6	(*6*)
(*p*-Fluorophenyl)-methylcarbinol	C_8H_9OF	13.55	13.63	0.6	(*5*)
o-Fluorotriphenylmethane	$C_{19}H_{15}F$	7.24	7.19	0.6	(*3*)
o-Fluorotriphenylchloromethane[b]	$C_{19}H_{14}ClF$	6.40	6.37	0.5	(*3*)
Benz-*p*-fluoroanilide	$C_{13}H_{10}ONF$	8.82	8.79	0.3	(*31*)
Methyl fluoropyruvate	$C_4H_5O_3F$	15.82	15.92	0.6	(*22*)
1,1-Di-(*p*-bromophenyl)-2,2,2-trifluoroethane[c]	$C_{14}H_9Br_2F_3$	14.46	14.57	0.8	(*19*)
p-Fluoroacetophenone-dithioethylene-ketal	$C_{10}H_{11}FS_2$	8.86	8.82	0.4	(*4*)

[a] Average of at least three determinations.
[b] Found 11.92% chlorine (calcd. 11.96%).
[c] Found 40.19% bromine (calcd. 40.56%).

The residue of the combustion accelerator, if used, does not affect the result adversely. Obviously, if other halogens are to be determined simultaneously, no potassium perchlorate is added. Phosphorus in organic fluoro compounds interfered with the determination, and a modification of the method suitable for this type of compound is in preparation.

The calibration curve is linear only in the range corresponding to 0.1 to 0.3 mg. of fluoride ion per 10 ml. of final solution. Solutions of a higher fluoride concentration have to be diluted accordingly.

In contact with alkali (about 8% sodium hydroxide solution), the Amberlite IR-112 changes its color from orange to brown, thus indicating the progress of the front of the alkaline solution and making the control of the activity of the column fairly simple. It should be borne in mind that the column warms up when it comes in contact with an alkaline solution. The cation exchanger Amberlite IR-120, which was tried first, is not applicable to the *determination* of fluoride ion as it swells in presence of an alkali and makes percolation impossible.

ACKNOWLEDGMENT

The authors are indebted to Ernst D. Bergmann for his interest in this work, to Wolf Bodenheimer for his helpful suggestions, and to Felix Deutsch for the construction of the peroxide bomb. They would also like to express their gratitude to Rohm & Haas, Philadelphia, Pa., and their representative in Israel, Hadbarah, Ltd., Tel-Aviv, Israel, for providing the samples of Amberlite ion exchanger.

The experiments were carried out under the auspices of the Scientific Department, Israel Ministry of Defence.

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RECEIVED for review April 11, 1955. Accepted September 7, 1955.

Coulometric Titration of Arsenic(III) Solutions with Cerium(IV)

Spectrophotometric End-Point Detection

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The titration of arsenious acid with ceric sulfate has long been an excellent method for standardizing ceric solutions and for determining macro quantities of arsenic in acid solutions. No previous attempt has been made to test the applicability of this classical titration to coulometric ceric oxidimetry. The otherwise slow reaction is catalyzed by a trace of osmium tetraoxide. Spectrophotometric end-point detection with an unmodified Beckman DU instrument is shown to be accurate, providing the limitations of the method are understood. The end-point procedure depends upon the strong ceric absorbance maximum at 320 mμ. The method thus consists of detecting the absorbance due to excess ceric ion and extrapolating to zero absorbance. There is no absorption by the components of the solution until the equivalence point is passed. The results compare favorably with those obtained in the titration of reduced arsenic solutions with electrically generated iodine, bromine, and chlorine in which amperometric, potentiometric, or photometric end-point procedures were used.

Several investigators have reported the determinations of reduced arsenic solutions by electrically generated reagents. Swift and others have used iodine (*13*), chlorine (*6*), and bromine (*11*) to determine arsenite solutions coulometrically. Amperometric end-point procedures were employed throughout by these investigators. Everett and Reilley (*5*) have described a method for determining arsenite with electrically generated iodine, utilizing spectrophotometric measurements of excess iodine at 342 mμ to determine the end point. An automatic coulometric titrator with photometric indication in the visible region has been shown applicable to the arsenite-iodine reaction (*15*).

On a macro scale potentiometric indication has been described as accurate for the titration of arsenite with externally generated iodine, bromine, and chlorine (*12*). A semimicro method with electrically generated permanganate is reported to give reproducible results when *o*-phenanthroline ferrous sulfate is employed as indicator (*14*).

Electrically generated cerium(IV) in sulfate media has been used to determine ferrous ion coulometrically. The end points can be determined potentiometrically (*9*), or with the sensitive end-point procedure of Cooke, Reilley, and Furman (*2*). Hydroquinone, *p*-aminophenol (*7*), uranium(IV) (*8*), and ferrocyanide (*4*) have also been successfully determined coulometrically with cerium(IV). The end points in these determinations were found using the "sensitive end-point" procedure.

The coulometric ceric–arsenious acid reaction has not hitherto been reported, nor has a spectrophotometric end-point procedure been attempted previously for coulometric ceric titrations. This investigation was initiated to test the feasibility of applying spectrophotometric end-point detection to coulometric ceric titrations. The method was tested by titrating microgram quantities of arsenious acid, which in itself is a new application for coulometric ceric oxidimetry.

Bricker and Sweetser (*1*) describe a spectrophotometric method for determining microgram quantities of arsenious acid by direct titration with ceric sulfate solutions as dilute as $4 \times 10^{-4}N$. Their background medium was $1N$ sulfuric acid. The work described here is similar, but a limitation was encountered on their method when it was applied to coulometric procedures. In coulometric generations of ceric ion from a saturated cerous solution, $1N$ in sulfuric acid, a definite reagent blank was found which was not reproducible. This lack of reproducibility is very likely due to slow reaction of ceric ion with the oxidizable species present in the cerous sulfate or acid and solvent. The blank was eliminated by generating ceric ion to an arbitrary absorbance, allowing the impurities to react, adding an excess of arsenious acid, and titrating the excess in the usual manner. The current-time product for the blank plus excess arsenious acid is then subtracted from the first determination. Several subsequent samples may be analyzed in the same generating medium.

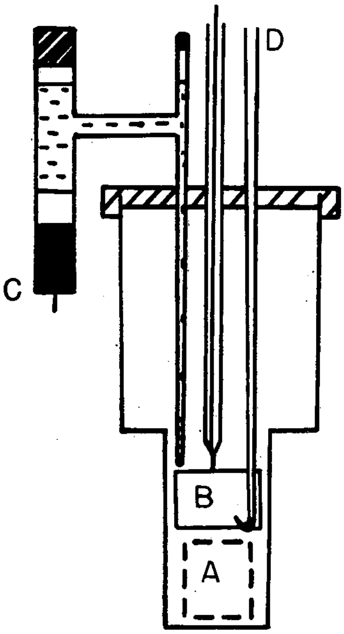

Figure 1. Titration cell

A. Light path
B. Generating electrode, 2 × 2 cm.
C. Lead amalgam isolated electrode
D. Capillary inlet tube for gas stirring

APPARATUS

All work described here was done with a Beckman DU spectrophotometer. The titration cell is shown in Figure 1; it is identical with that described by Bricker and Sweetser (*1*). There was sufficient space above the light path to accommodate the necessary electrodes while making it possible to minimize the solution volume. A felt cover with a slit kept stray light from the cell chamber; nitrogen gas passing through a capillary stirred the solution. No galvanometer unsteadiness was experienced if bubbles did not enter the light path.

The electrical current was supplied by a bank of 45-volt radio B batteries delivering a total of 225 volts. Constancy of current was obtained at the higher current levels by using additional batteries in a series-parallel arrangement. The current was meas-

Table I. Titration of Arsenic(III) with Cerium(IV)

Arsenic, γ		Error		Generator Current, Ma.
Taken	Found	γ	%	
932.6	936.7	4.1	+0.44	4.718
	932.1	0.5	−0.05	4.708
	932.9	0.3	+0.03	4.683
	935.1	2.5	+0.27	4.820
	933.1	0.5	+0.05	4.800
Av.	934.0	+1.4	+0.15	4.716
373.0	372.7	0.3	−0.08	4.799
	374.4	1.4	+0.37	4.790
	371.6	1.4	−0.37	4.788
Av.	372.9	−0.1	−0.03	4.792
145.5	145.1	0.4	−0.27	1.051
	145.5	0.0	+0.00	1.050
	145.1	0.4	−0.27	1.050
	146.0	0.5	+0.34	1.053
	146.5	1.0	+0.69	1.052
	145.4	0.1	−0.07	1.051
	145.9	0.4	+0.27	71.050
Av.	145.6	+0.1	+0.15	1.051
110.8	112.1	1.3	+1.2	0.4710
	112.2	1.4	+1.3	0.4710
	111.6	0.8	+0.7	0.4710
	110.4	0.4	−0.4	0.4721
	111.2	0.4	+0.4	0.4721
	111.2	0.4	+0.4	0.4721
Av.	111.4	+0.6	+0.6	0.4715
73.96	75.13	1.2	+1.6	0.4710
	74.22	0.3	+0.4	0.4710
	74.22	0.3	+0.4	0.4710
	73.88	0.1	−0.01	0.4700
	74.61	0.7	+0.9	0.4700
	73.82	0.1	+0.01	0.4700
	74.28	0.3	+0.4	0.4700
	74.95	1.0	+1.3	0.4700
Av.	74.39	+0.5	+0.6	0.4704
58.18	59.40	1.2	+2.1	1.055
	59.77	1.6	+2.7	1.054
	58.91	0.7	+1.2	1.054
	59.31	1.1	+1.9	1.054
	58.32	0.1	+0.2	1.054
	57.93	0.3	−0.5	1.053
Av.	58.96	+0.8	+1.4	1.054

ured by taking the voltage drop across the resistance of an Otto Woulf decade box with a Leeds & Northrup No. 7655 potentiometer. An S-6 clock, Standard Electric Time Co., having a start-stop error of 0.01 second, was used to measure the generation time. The circuitry was similar to that described by Cooke, Reilley, and Furman (*3*). A dummy resistance of 1000 ohms was added to the circuit shown there to eliminate current fluctuation on interruption.

The generating anode was a piece of bright platinum-iridium foil (10% iridium) measuring 2 × 2 cm. The isolated cathode was a lead amalgam-lead sulfate-1*N* sulfuric acid half cell. The side arm plug was constructed of paper saturated with 1*N* sulfuric acid (*7*).

REAGENTS

The generating medium was a saturated solution of cerous sulfate in 1*N* sulfuric acid prepared from the reagent grade octahydrate (G. F. Smith Chemical Co., Columbus, Ohio). The concentrated sulfuric acid was distilled in an effort to minimize the blank. The arsenious acid was prepared from National Bureau of Standards primary standard arsenious oxide in the usual manner to obtain 0.1*N* stock solutions. Because arsenious oxide is a primary standard, the normalities were calculated by weight and checked by titration with standard 0.09979*N* potassium permanganate, using osmium tetroxide as catalyst. The titrimetric standardizations agreed with the calculated values to an average deviation of 3 parts in 10,000 for six titrations. Aliquots of a stock solution were measured out with calibrated glassware and diluted to volume with 1*N* sulfuric acid to obtain solutions of the correct normality. These dilute stock solutions were made up daily. Portions of diluted stock were transferred by calibrated pipet directly into the titration cell.

The osmium tetroxide (*G. F. Smith Chemical Co.*) was a 0.01*M* solution, 0.1*N* in sulfuric acid.

PROCEDURE

Approximately 50 ml. of saturated cerous sulfate generating solution were placed in the titration cell. The nitrogen was turned on to mix and deaerate the contents of the cell. The wave length was set at 320, 360, or 375 mμ, depending on the current level, and the absorbance dial was set at 0.200. With the dark current balance at zero, the photocell was exposed and the slit width adjusted to zero galvanometer deflection. The sensitivity knob was maintained at a constant position 3 revolutions from its counterclockwise limit. The current was applied and the reagent blank measured. After generating ceric ion until the slope of the absorbance-time plot appeared constant, an aliquot of arsenious acid sample and 1 to 2 drops of osmium tetroxide solution were added and the contents of the cell mixed thoroughly. The absorbance was noted and sample *A* titrated. Subtracting the current-time product for the blank from the product for sample *A* should result in the theoretical product for the amount of arsenious acid present. However, it was not found possible to determine a reliable blank correction for any given amount of generating medium; therefore, it was found necessary to generate ceric ion to an arbitrary absorbance, add an excess amount of arsenious acid, and generate ceric ion to an end point determined by a plot of absorbance *vs.* time. Then, upon adding a succession of samples and titrating coulometrically, it was found that good results were obtained by integrating the current-time values between any two successive end points after the first.

The initial absorbance setting must be set arbitrarily at some positive value—e.g., 0.200—because the absorbance falls due to dilution after each new addition of arsenious acid. Six or seven 2-ml. samples could be analyzed in the same generating solution before the slope of absorbance *vs.* time became too flat to allow an accurate determination of the end point.

Figure 2 shows part of a typical run. It is possible to take the initial absorbance after each addition of sample as the horizontal portion of the graph because nothing present in the solution absorbs light at the wave length employed until the end point is passed. At 360 and 375 mμ the drop in absorbance through dilution was negligible until after three 3-ml. samples had been added.

RESULTS

The results are presented in Table I. No results are reported for quantities of arsenic less than 58 γ, although smaller samples could be done by this method at lower generating current levels. The limit of sensitivity is governed by the relatively smaller absorbance increase in lower generating current levels leading to

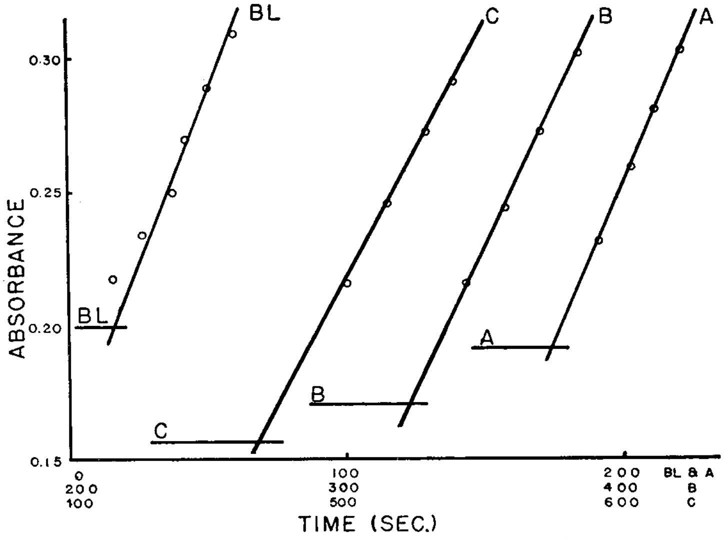

Figure 2. Titration graphs for 58.18 γ of arsenious acid

BL is attempted titration of blank; *A*, *B*, and *C* correspond to titration of known quantities of arsenic(III).

inaccuracy in finding the true end point. Larger samples are possible but the simple battery arrangement used here limited usable constant current level.

DISCUSSION

Accurate results are attainable with this method if care is taken to oxidize the trace impurities present. In previous work on ceric oxidimetry the effect of trace impurities has not been evident, largely because the sensitive end-point procedure of Cooke and others (*2*) has been applied (*4*, *7*, *8*). Any reagent blank is eliminated in this method by the initial generation of ceric ion to the preset end-point potential as determined by a semimicro potentiometric titration.

Although several papers have appeared on the use of photometric techniques to determine the end points in coulometric titrations, this paper is the first to deal with the use of the high ceric absorbance in the near ultraviolet region. The determinations reported here, some unpublished data on brominations done in this laboratory (*10*), and the recent work of Everett and Reilley (*5*) represent the first in which advantage has been taken of strong absorbances in the near ultraviolet to give sensitive end points.

With few exceptions, coulometric methods show an absolute error of the general order of 1 γ. Under exceptionally favorable circumstances significant results may be obtained in the submicrogram range, as in the titration of permanganate with ferrous ion (*13*). Contributing factors which tend to limit the lowest range for any particular method appear to be (1) unfavorable reaction kinetics, (2) trace impurities in reagents, and (3) possible consumption of the oxidant to form the initial oxide layer on the inert generating electrode or reduction of this oxide layer by the reductant.

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Received for review November 19, 1955. Accepted February 1, 1956.

Table Suitable for Mounting a Microchemical Balance

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A table suitable for mounting a microchemical balance in an area in which there are vibration disturbances has been constructed, the essential parts being a reinforced concrete block on deflected springs. The springs reduce transmission of the vibrations present, while the block acts as an inertia mass to reduce the movement that is caused by normal manipulation of the balance. Oscillograph recordings showed that the system has an isolation efficiency of about 93%. To prevent the operator from coming in contact with the resiliently mounted system, except through normal manipulation of the balance, protective woodwork was incorporated. Ten microchemical balances (including four different makes), mounted on tables of this type, have precisions which range from 1.1 to 2.6 γ, in spite of the fact that they are located on the floor above a manufacturing area.

The importance of a suitable table for mounting a microchemical balance has been stressed by a number of authors (*1–3*, *5*, *8–12*, *14*, *18*, *21*, *23*, *24*), because it is recognized that vibrations and shocks from external sources cause damage to the knife edges.

Pregl (*18*, *21*), Emich (*5*), and Alber and Harand (*1*, *2*) stressed the generous use of lead as a means of absorbing vibrations. Various types of spring systems (large and small, damped and undamped) (*7*, *10*, *12*, *16*, *17*, *22*); two heavy plates separated by dry sand (*26*); and rubber (*1–3*), including balls (*13*, *14*), have been used. Tables constructed of cork, brick, wood, lead, and stone slabs have proved very satisfactory in the laboratories of this company (*23*, *24*) as well as in others (*6*, *19*, *25*). However, during the process of expanding this company's laboratories, a new location was selected in which there is considerable vibration caused by the presence of a number of centrifuges and pumps.

A table of the type used in the old laboratories, as well as various modifications, did not absorb vibrations to the desired extent. Consulting engineers, specializing in vibration problems (Korfund Co., Inc., 48-15 32nd Place, Long Island City 1, N. Y.), advised that the use of springs, which would reduce transmission of the vibrations, would offer the best means of isolation. Balances thus mounted are, however, in danger of being damaged owing to movement of the system caused by the force exerted on it by the operator during normal operation of the balance. To minimize this effect, each balance was placed on a separate reinforced concrete inertia block mounted on deflected springs, the block acting as an inertia mass to reduce this movement. It is desirable to have the mass as large and the center of gravity of each block as low as practical, with the springs spaced as far apart as possible for increased stability. Each location presents its own vibration problem which should be solved independently, but the same principles are applicable and the selection of the proper combination of springs and inertia mass should make it possible to construct a suitable table. The manner of solving the problem in these laboratories will serve as an example.

Vibration isolation depends upon the ratio of the frequency of the disturbing vibration, F_D, and the natural frequency of the isolation mountings, F_N, the efficiency being expressed by the formula (*15*),

$$\%\ \text{efficiency} = 100\left(1 - \frac{1}{(F_D/F_N)^2 - 1}\right)$$

where

$$F_N = 188\sqrt{\frac{1}{D}} \quad \text{cycles per minute}$$

and D, the deflection of the loaded isolation mounting, is given by

$$D = \frac{\text{load}}{K}$$

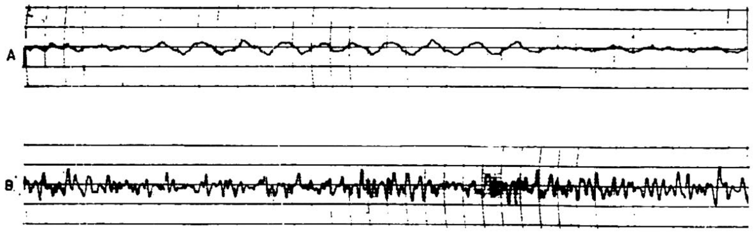

Figure 1. Oscillograms

A. Inside mounted balance
B. On floor of balance room

K, the spring constant, is the number of pounds required to make the mounting deflect 1 inch. If a force is applied to the loaded mounting to give a slight additional displacement and is then released, as during normal operation of the balance, the mounting will oscillate at a definite frequency (the natural frequency, F_N) which depends upon the static deflection of the mounting shown in the equation above. From the formula for the efficiency, it can be seen that a mounting having a natural frequency that will isolate the lowest expected disturbing frequency with a particular efficiency will isolate higher frequency disturbances with a higher efficiency.

In these laboratories, the closest source of disturbance is six 40-inch basket-type centrifuges on the floor beneath the balance room, which operate at 900 r.p.m. If unbalanced, the centrifuges would generate 900 cycles per minute disturbances; therefore, it was decided to protect the balances against these. Nine balances were to be mounted in the same room and, for safety reasons, the total weight of the tables had to be limited to 9000 pounds. This includes the weight of the blocks, springs, and protecting woodwork necessary to prevent the systems from being touched anywhere at any time except through the balances during operation. The weight of the springs per balance is slightly less than 100 pounds and the enclosing woodwork is approximately the same; therefore, the maximum weight of the reinforced concrete inertia block, per balance, was set at 800 pounds. Mountings (or isolators) were selected suitable for a 200-pound load per isolator, with approximately 1.2 inches deflection and a natural frequency of 172 cycles per minute (*15*). Four of these, uniformly loaded under an 800-pound block, would have, according to the formula above, a theoretical isolation efficiency of 96% for disturbances of 900 cycles per minute.

After the construction was completed, a number of oscillograph recordings were taken both on the floor and inside each of the mounted balances, as a means of determining the isolation efficiency of the systems. In the latter cases they included recordings while the balances were undisturbed and during normal operation. The oscillograms were taken by the Korfund Co., Inc., with an inertia-type vibration pickup and oscillograph (Models BL-301, and BL-211, respectively, Brush Development Co., Cleveland 14, Ohio) and were analyzed by the method prescribed by the instrument manufacturer (*4*). Figure 1 shows typical oscillograms taken inside the mounted balance and on the floor. On these charts, the horizontal interval between the vertical graduations denotes an elapsed time of 1/25 second (25 scale divisions per second). The middle portion of Figure 1, *A*, shows the presence of some oscillation of relatively high amplitude and low frequency, caused by one or more of the disturbances referred to above, which is damped out in less than 1 second. Analysis showed that during the disturbed condition the displacement of the table was 0.000047 inch at 12 c.p.s. (probably one of the natural frequencies of the isolated system, although not the vertical natural frequency of the springs). Analysis of Figure 1, *B*, showed that the displacement of the floor was 0.0000033 inch at a predominance of 70 c.p.s.

The accelerations above were calculated by the formula

$$\text{Acceleration (feet per second/second)} = 0.0511 \times D \times F^2 \times 32.2$$

where D = displacement in inches, and F = frequency in cycles per second. Results for the table and the floor were, respectively, 0.011 foot per second² and 0.027 foot per second². Therefore, during the disturbed condition, the displacement of the table was about 14 times that of the floor, but the acceleration of the table was about 40.7% of that of the floor, the latter being the more important for protecting the balances (*15*). However, during the time that the table was not disturbed, represented by the portions of Figure 1, *A*, on the left and right-hand sides, the displacement of the table was about $^1/_{14}$ that of the floor and the acceleration of the table was about 7% of that of the floor, which represents the residue of the vibration getting through the springs to the table. In other words, the efficiency under these conditions was about 93%.

Upon being displaced, the tables must return to their original

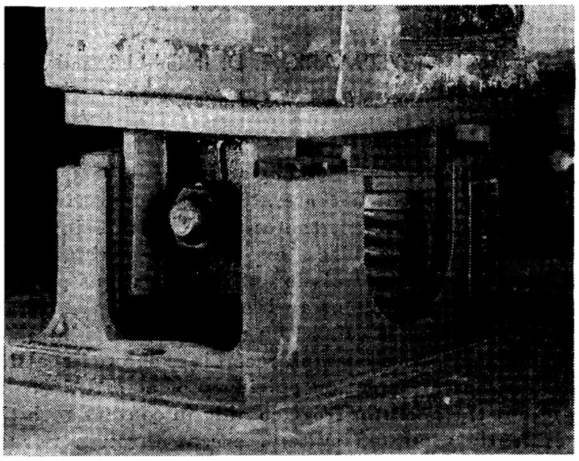

Figure 2. Close-up of isolator

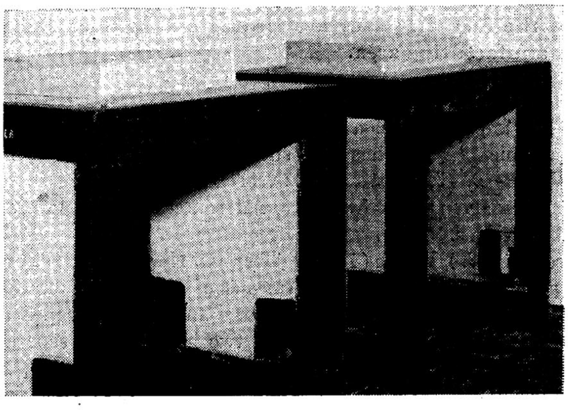

Figure 3. Inertia blocks mounted on isolators

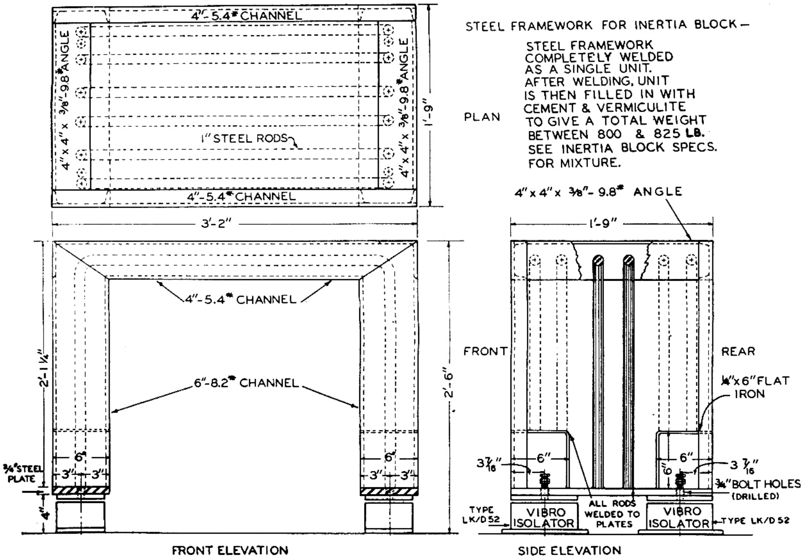

Figure 4. Details of construction of steel framework for inertia block

positions or the precision of the balances would be affected. To prove this point, zero readings of the balances were made, the balance knife-edges were supported in the normal manner, and the balance table was pushed so as to cause a displacement of approximately 1 inch. Then the tables were allowed to return to their natural positions. Zero readings of the balances were again made and it was found that these were within acceptable limits of reproducibility (*20*, *24*) of those obtained before disturbing the tables. The microchemical balances used in these tests included four Wm. Ainsworth & Sons, Inc. (Denver, Colo.), Type FDI; four Christian Becker (Clifton, N. J.), Model EM1; one Paul Bunge (Hamburg, Germany), Model 25MP; and 1 Mettler (Mettler Instrument Corp., Hightstown, N. J.), Model M5 (late 1955 version). The precision (*24*) of these mounted balances (at no load, except the Mettler which has a constant load) ranged from 1.1 to 2.6 γ, the figure for each being better than that claimed by the individual manufacturers.

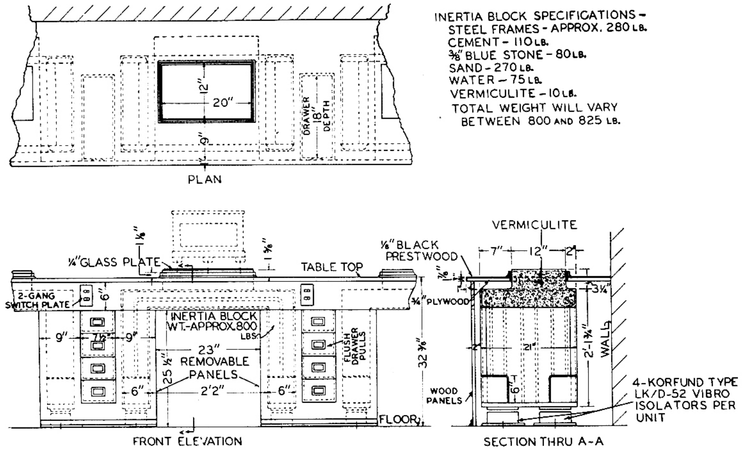

Figure 5. Details of construction of inertia block and surrounding woodwork

DETAILS OF CONSTRUCTION

Isolators. The isolators are commercially available (*15*). Korfund Type LK/D-52 Vibro-Isolators were recommended by the manufacturer after consideration of the various pieces of equipment causing vibrations in the building. A set of four isolators is required for each reinforced concrete inertia block, one at each of the four corners. Figures 2 and 3 show the isolators in place.

Reinforced Concrete Inertia Blocks. One of these, weighing approximately 800 pounds, is used for each balance. Figure 3 shows the blocks and Figures 4 and 5 give the details of construction. Dimensions of the plateau (12 by 20 inches), Figure 5, may be varied to accommodate the balance in question. Those shown are suitable for the Ainsworth, Becker, and Bunge balances. A plateau measuring 15 by 16 inches is suitable for a Mettler microchemical balance.

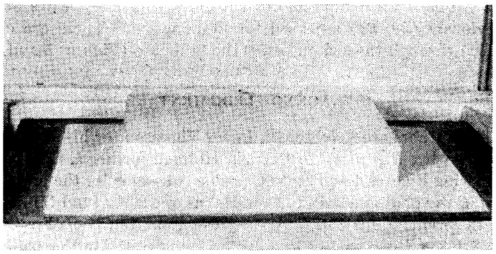

Figure 6. Inertia block encased in woodwork before table top is put in place

Woodwork. The woodwork construction of the table is designed to protect the inertia block from being touched by the operator. The details of construction are given in Figure 5. A clearance of 1 inch exists between the inertia block and the woodwork at all points. This is shown in Figure 6, a photograph taken during construction before the table top was added. The table top has an opening to allow the small concrete plateau, measuring 12 by 20 inches, to protrude through it, but not touch it. Upon this plateau rest a glass plate $^1/_4$ inch thick and, finally, the balance. A small molding is attached to the table top to prevent the plateau from coming in contact with notebooks and other objects. Removable panels are used in the knee space, so that, if necessary, adjustment of the iso-

Figure 7. Section of finished balance table

lators can always be made with the minimum of difficulty. Figure 7 shows a part of the finished table, which accommodates nine balances

Lighting. Individual fluorescent fixtures of the type described previously (*23, 24*) are used for illumination. These are controlled through the switch below the table top (Figures 5 and 7).

ACKNOWLEDGMENT

The authors are indebted to James Thomson for his assistance in preparing Figure 4; to Patricia Hillman for her assistance in preparing Figures 4 and 5; to Carmine Auricchio for the preparation of the photographs; to Jack Harris of the Korfund Co., Inc., for the oscillograms and analysis of them; and to Hans Frey and the Mettler Instrument Corp. for supplying the Mettler balance used in the tests.

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Received for review October 25, 1955. Accepted February 6, 1956.

Coulometric Titration of Iron(III) with Electrolytically Generated Iron(II)–Ethylenediamine Tetraacetate

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Conditions for coulometric titrations with electrolytically generated iron(II)–ethylenediamine tetraacetate are described. Automatic and manual titrations of iron(III) gave satisfactory results. The redox system iron(III/II)–EDTA and iron(III/II) was investigated potentiometrically in order to select the optimum conditions.

SCHWARZENBACH and Heller (*11*) pointed out that the strikingly strong reduction power of mixtures of ferrous salts with (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid, EDTA, or H_4Y) may lead to practical applications. They determined the redox potential over a pH range from 2.4 to 8.3, by means of the potentiometric titration curves of ferrous ethylenediamine tetraacetate with chlorine or bromine, and found it to have a constant value of +117.2 mv. (*vs.* normal hydrogen electrode) from pH 4 to 6. At higher and lower pH the redox potential depends strongly on pH (Figure 1). The reduction power is similar to titanous (E° = ca. + 0.1 volt *vs.* normal hydrogen electrode) but applicable over a different pH region. Kolthoff and Auerbach (*5*) investigated the same system polarographically in a pH range from 1 to 11 and obtained half-wave potentials deviating by only a few millivolts from the values reported by Schwarzenbach and Heller.

In analytical chemistry, iron(II)-EDTA has been used for determinations of iodine (*7*), silver (*6*), and copper (*2*).

Coulometric titrations are especially valuable when the titrant is an unstable substance. As iron(II)-EDTA is very sensitive to air, it seemed desirable to work out conditions for the electrolytic generation of this reagent.

Iron(II)-EDTA was generated cathodically at a platinum electrode from iron(III)-EDTA by the reaction:

$$FeY^- + e \rightarrow FeY^{--}$$

The resulting iron(II)-EDTA titrant then reacts in the bulk of the solution with the iron(III) sample:

$$FeY^{--} + Fe^{+++} \rightarrow FeY^- + Fe^{++}$$

A more detailed study was made on the effect of pH on this titration reaction in order to find the optimum pH condition.

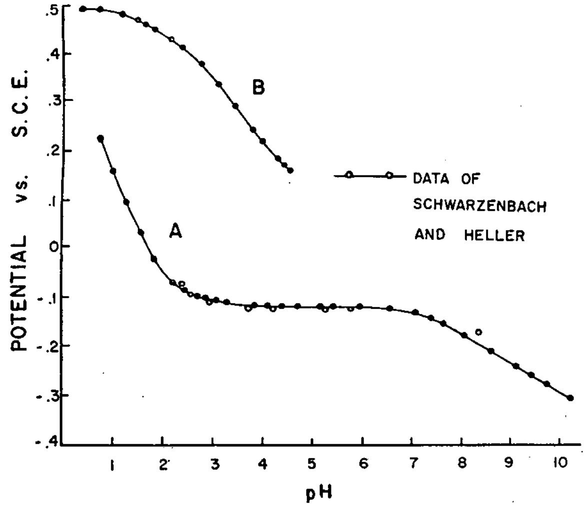

Figure 1. pH–potential diagram

A. Iron(III/II)–ethylenediamine tetraacetate redox system
B. Iron(III/II)–redox system (in excess acetate)

For this purpose the redox potential of the iron(III)-EDTA–iron(II)-EDTA has been measured potentiometrically as a function of pH, as is shown in curve *A*, Figure 1. The values agree well with the points reported by Schwarzenbach and Heller (*11*).

The reduction potential of the iron(III)-EDTA–iron(II)-EDTA system becomes more negative in alkaline solution, but solutions more alkaline than pH 10 are unstable and slowly form precipitates of iron(III) hydroxide (*11*).

At pH values lower than 2 the redox potential changes rapidly in a positive direction (230 mv. per pH). Although the slope of this rise corresponds fairly closely to the participation of 4 protons in the potential-determining equilibrium, the process is actually a mixed one, in view of the two equilibria (*10*):

$$\begin{array}{ll} HFeY \rightarrow H^+ + FeY^- & pK = 1.4 \\ H_4Y \rightarrow H^+ + H_3Y^- & pK = 2.0 \end{array}$$

In order to obtain a strong reducing power it is desirable to work at pH values higher than 2. Therefore, partial neutralization of the free acid formed in the preparation of iron(III)-ethylenediamine tetraacetate is necessary.

$$Fe^{+++} + H_2Y^{--} \rightarrow FeY^- + 2H^+$$

Partial neutralization with sodium hydroxide, however, leads to an opalescence, attributed to the precipitation of iron(III) hydroxides in the vicinity where the drops of base are added. This opalescence clears only very slowly after stirring, but could be entirely avoided by neutralizing the solution with sodium acetate.

On the other hand, an equally important consideration is the effect of pH on the reduction potential of the substance to be titrated. Acetate ions complex ferric ions more tightly than ferrous ions, and therefore shift the redox potential of this system to more negative values, as shown in curve *B*, Figure 1. The curve represents the potential of an iron(III)–iron(II) solution containing excess acetate as a function of pH. At pH values greater than 3, basic acetates precipitate. Cheng, Bray, and Kurtz (*3*) found that at pH values greater than 3 the recovery of iron as determined by titration with EDTA was no longer 100%. Even though a stock solution of ferric ion in the present work appeared clear at pH 4 (a weak Tyndall beam, however, existed), incomplete recovery was found.

In summary, Figure 1 shows that in order to obtain a large potential break at the end point (corresponding to the difference between curves *A* and *B* in Figure 1) and to avoid the formation of basic precipitates, the titration is carried out best at pH 2 to 3. The iron(III)-EDTA stock solution was consequently neutralized with sodium acetate to the desired pH of 2.5 (final concentration 0.1*M*).

Iron(II)-EDTA is very easily oxidized by air (*4*, *5*) and therefore air must be excluded carefully. Iron(III)-EDTA is decomposed by sunlight (*4*) and care has to be taken in this respect.

EXPERIMENTAL

Cell. The cell shown in Figure 2 was used.

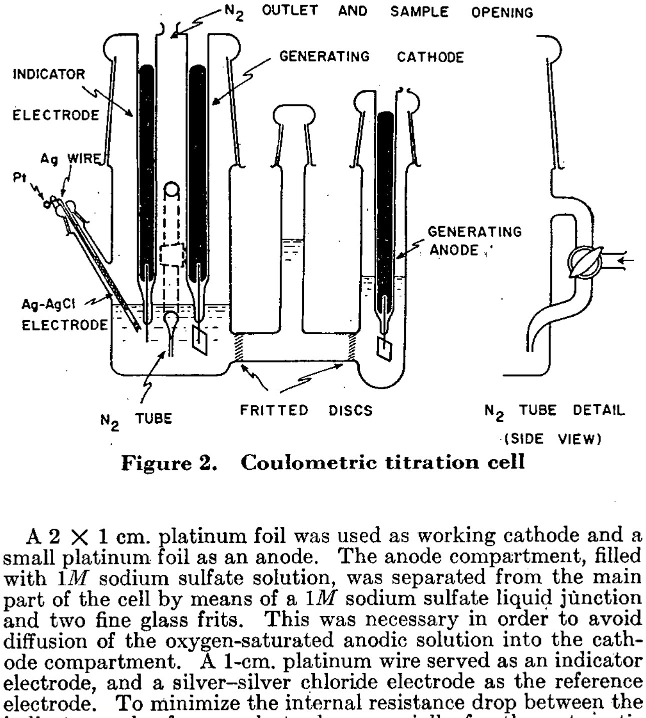

Figure 2. Coulometric titration cell

A 2 × 1 cm. platinum foil was used as working cathode and a small platinum foil as an anode. The anode compartment, filled with 1*M* sodium sulfate solution, was separated from the main part of the cell by means of a 1*M* sodium sulfate liquid junction and two fine glass frits. This was necessary in order to avoid diffusion of the oxygen-saturated anodic solution into the cathode compartment. A 1-cm. platinum wire served as an indicator electrode, and a silver–silver chloride electrode as the reference electrode. To minimize the internal resistance drop between the indicator and reference electrodes, especially for the automatic titrations, the electrodes in the indicator circuit were kept close together and away from the generator electrode circuit.

Constant Current Supply. For small currents, a supply previously described (*9*) was used which furnished currents from 0.005 to 4 ma. Current measurements with an accurate potentiometer circuit (Leeds & Northrup student potentiometer) showed that current fluctuations did not exceed 0.1%. For the titration of the larger amounts of iron, a supply (*8*) which gave one defined current of larger magnitude (43.30 ± 0.10 ma.) was used.

REAGENTS

Iron(III)-EDTA Solution. To a solution of iron(III) chloride (Merck) an exactly equivalent amount of a disodium dihydrogen ethylenediamine tetraacetate solution was added and the resulting solution was partially neutralized to pH 2.5 by an equivalent amount of sodium acetate. The exact titer of the respective solutions was determined relative to each other by electrometric

Table I. Titration of Iron(III) by Electrolytically Generated Iron(II)-EDTA

Current, Ma.	Iron Taken, Mg.	Iron Found, Mg.	Deviation	
			Mg.	%
43.30	17.52	17.44	−0.08	−0.5
		17.52	0.00	0.0
		17.62	+0.10	+0.6
		17.42	−0.10	−0.6
		17.57	+0.05	+0.3
		17.47	−0.05	−0.3
43.30	7.01	7.04	+0.03	+0.4
		7.09	+0.08	+1.1
		7.02	+0.01	+0.1
		7.02	+0.01	+0.1
3.862	2.63	2.64	+0.01	+0.4
3.864		2.67	+0.04	+1.5
3.880		2.67	+0.04	+1.5
2.886		2.64	+0.01	+0.4
2.002	1.402	1.404	+0.002	+0.1
2.001		1.398	−0.004	−0.3
2.002		1.404	+0.002	+0.1
2.002		1.406	+0.004	+0.3
1.997	1.402	1.406	+0.004	+0.3
1.997		1.400	−0.002	−0.1
1.995		1.405	+0.003	+0.2
1.993		1.406	+0.004	+0.3
1.1424	0.701	0.700	−0.001	−0.1
1.1420		0.703	+0.002	+0.3
0.9992	0.701	0.689	−0.012	−1.7
0.9992		0.697	−0.004	−0.6
0.9991		0.709	+0.008	+1.1
0.9991		0.694	−0.007	−1.0
0.5008	0.280	0.271	−0.009	−3.3
0.5022		0.272	−0.008	−2.9
0.5027		0.725	−0.005	−1.8
0.5022		0.275	−0.005	−1.8

titrations. The solution was then diluted to give a final concentration of 0.1M iron(III)-EDTA. The solution should be allowed to stand overnight before use, because its blank value changes in the beginning.

Nitrogen. For bubbling oxygen from the solutions, oxygen-free nitrogen (Airco, Seaford grade) was used, being bubbled first through water to saturate the nitrogen with water vapor.

Ferric Chloride Solutions. The titer of the iron(III) chloride stock solution was determined by potentiometric titration with a standard EDTA solution (*1*). The solutions used for the coulometric titrations were subsequently made up by dilution of this stock solution.

DETERMINATION OF END POINT

The potential of the platinum indicator electrode was measured against a saturated calomel electrode or silver–silver chloride with a Leeds & Northrup pH meter (Type 7664), which in turn was connected to a Brown recorder in order to register the titration curves automatically. A chart speed of 0.5 inch per minute was used and the current so chosen that the length of chart paper for a titration curve was 8 to 10 inches.

When a current of 43 ma. was used, the titration curves were determined manually. For each point the current was temporarily interrupted, and the time (Gra Lab Micro timer, Type 202) and potential were noted. The potential in the vicinity of the end point required 5 to 10 seconds to reach equilibrium.

PROCEDURE

The cell was filled with 25 to 30 ml. of the iron(III)-EDTA solution (0.05M for titrations at any current up to 4 ma., 0.1M for the current of 43 ma.), and deaerated 10 minutes with nitrogen. A pretitration was carried out next after addition of a small but unmeasured amount of the solution to be titrated. The sample was then added and titrated. The distance between these two end points was then measured and taken to represent the titer of the sample. With the prescribed amount of iron(III)-EDTA background electrolyte four 5-ml. samples could be titrated one after the other before the cell was refilled with fresh background solution. During the titration the solution was vigorously stirred with a magnetic stirrer and a slow flux of nitrogen bubbles was maintained.

MEASUREMENT OF REDOX POTENTIALS

A solution was prepared containing 0.01M iron(II) ammonium sulfate, 0.01M iron(III) chloride, 0.02M disodium dihydrogen ethylenediamine tetraacetate, and 0.04M sodium acetate. The potential of this solution as a function of pH was measured with a platinum electrode, using a Leeds & Northrup student potentiometer. The pH was adjusted by addition of concentrated per chloric acid or sodium hydroxide and measured with a glass electrode.

The procedure for the iron(III)–iron(II) system was analogous, the measured solution containing:

0.01M iron(II) ammonium sulfate
0.01M iron(III) chloride
0.2M sodium acetate

RESULTS

The results obtained are listed in Table I. The outstanding sources of error were estimated to be the following:

	Automatic Titration, %	Manual Titration, %
Graphic determination of end point	±0.5	±0.15
Reproducibility of pipet	±0.1	±0.1
Accuracy of Fe^{+++} titer	±0.3	±0.3
Constant current supply	±0.1	±0.25
	±1.0	±0.8

A comparison of this error estimation with the experimental results shows that the deviations of a single titration in general are within these error limits, with exception of the titration of 0.28 mg.

According to their reduction potentials, permanganate, chromate, vanadate, and uranyl should be reduced by iron(II)–ethylenediamine tetraacetate. However, experiments showed that these ions could not be titrated under the conditions used. Chromate and permanganate destroyed the iron(III)–ethylenediamine tetraacetate by oxidation of the organic part of the ion, whereas the reaction with uranyl and vanadate proceeds too slowly for practical direct titration. A back-titration procedure might be effective, but was not tried.

ACKNOWLEDGMENT

This research was supported by the United States Air Force, through the Office of Scientific Research of the Air Research and Development Command.

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Received for review September 9, 1955. Accepted December 19, 1955.

Significance of Rates and Equilibria in Electroanalytical Chemistry—Correction

In the article "Significance of Rates and Equilibria in Electroanalytical Chemistry" [Anal. Chem. **27**, 1708 (1955)], in Figure 1 curves II and IV should be interchanged: Curve II should intersect the zero current line at A, and curve IV at C. In Equation 14 the first term in the denominator should read:

$$\frac{m_{\text{Ox}}}{k_{\text{Red}} f_{\text{Ox}}}$$

Joseph Jordan

Modified Joliot Apparatus for Study of the Electrodeposition of Radioactive Materials

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An apparatus for continuously recording the deposition or dissolution of radioactive material upon a radiation-transparent electrode during potential change is described. Deposition of the radioactive element is detected by an increase in the ionization current record of an ionization chamber adjacent to the electrode; dissolution of the deposit is accompanied by a decrease in the ionization current. Potential control of the cathode or anode is effected by a variable electronic potential controller. The reversibility of electrode potentials is determined by alternately making cathodic and anodic potential sweeps. For reversible systems, the cathodic inflections occur at the same potentials as the anodic inflections. The use of the apparatus for recording deposition and dissolution potentials of polonium in 4.7*N* hydrochloric acid is described.

CONVENTIONAL methods of investigating the electrochemical properties of materials with high specific radioactivity are limited by the interference of decomposition products produced by irradiation of the solvent. For instance, polonium-plated electrodes in contact with perchloric or sulfuric acids can be polarized by an external current source, either positively or negatively, with many times the coulombic equivalent of the polonium itself without permanently changing the electrode potential. Such behavior indicates that the potential is controlled by solvent decomposition products rather than by the polonium–polonium ion couple.

The method of Joliot (*2*) is designed to detect deposition of a radioactive material without interference caused by inactive decomposition products. By this method, concentration of radioactive material on the cathode (or anode) is indicated by means of an ionization chamber placed adjacent to the radiation-transparent electrode. An increase in ionization current as the electrode potential is made more negative indicates that radioactive material is concentrating at the cathode. The ionization current is independent of electrochemical reductions other than those of the radioactive substances themselves, while the cell currents, as measured by polarography, may include the electrolytic reduction of solvent decomposition products. Also, the measurement of ionization current rather than cell current may extend the range of detection of deposition in very dilute solutions.

Joliot's method has been modified so that the potential of a radiation-transparent electrode may be uniformly varied and the ionization current in the vicinity of the electrode continuously recorded. Inflections in the graphic record of the ionization current as a function of electrode potential indicate deposition or dissolution of radioactive material.

APPARATUS

The solution vessel shown in Figure 1 is fabricated from two truncated ball joints. An alpha-transparent gold or platinum foil, approximately 0.0001 inch thick, is mounted between the ball joints to serve both as the bottom of the solution vessel and as the cathode (or anode). With this arrangement, gas bubbles formed by irradiation of the electrolyte tend to rise from the bottom electrode rather than to remain on its surface and disrupt electrical contact between the solution and electrode. Contact with the potential-controlling circuit is made by means of a gold washer 0.010 inch thick in contact with the foil. Neoprene gaskets prevent leakage of liquid between the glass and metal surfaces, and the assembly is held together by a modified ball joint clamp. A platinum

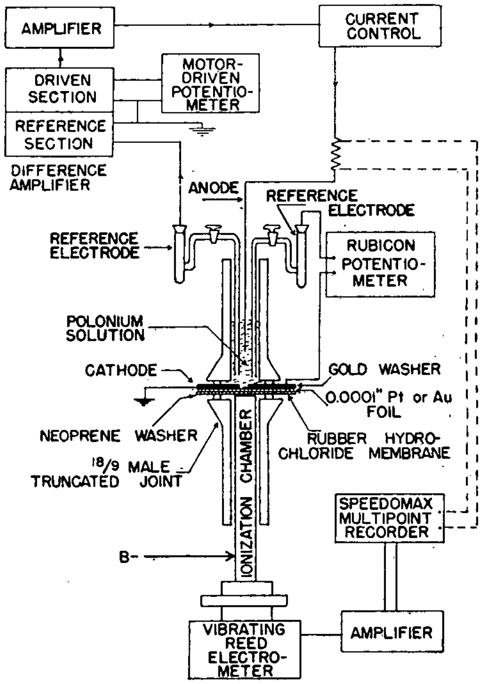

Figure 1. Potential control and deposition detecting apparatus

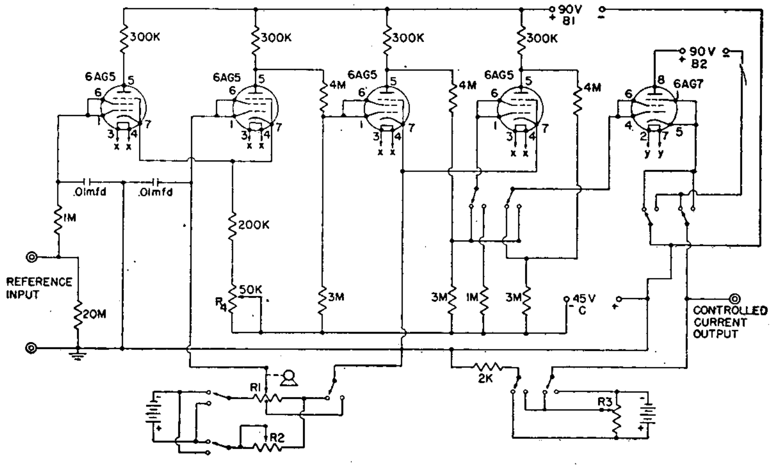

Figure 2. Electronic potential controller

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wire anode (or cathode) and reference electrodes are inserted through a rubber stopper at the top of the cell. To prevent radioactive contamination in working areas, the cell is enclosed in a hood unit equipped with glove ports. The section below the ball joint extends through the floor of the hood, so that the ionization chamber can be inserted from below.

In studying the deposition behavior of noble radioactive elements, such as polonium, stable anodes, such as mercury, cannot be used, since spontaneous reduction of polonium occurs at the mercury surface. An inert wire anode (or cathode) and an electronic potential controller (Figures 1 and 2) are therefore used to control the alpha-transparent electrode potential.

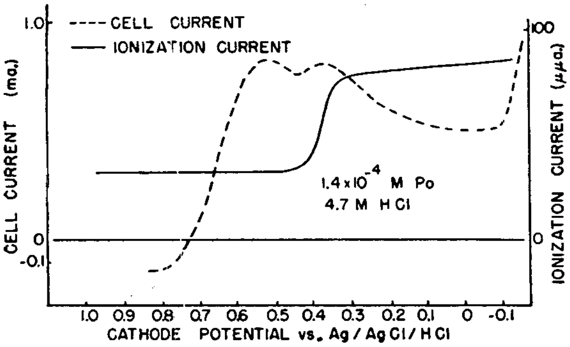

Figure 3. Ionization current and cell current relationship

The electronic potential controller is a modification of an early version of the controllers used to maintain a constant solution to cathode potential during electrodeposition (*1*). The fixed reference potential is replaced by a variable potential from a motor-driven potentiometer, *R*1; a phase inverter stage is added to permit anodic-potential sweep; and a variable bias, *R*3, is added to ensure maximum linearity of operation for V_5. The nine single-pole double-throw switches are shown in anodic-potential sweep position; they may be ganged for ease of control. *R*1 is a high-resistance Kohlrausch slide-wire. The synchronous motor drive is provided with several speeds and a reversing gear. *R*2 permits adjustment of the voltage span covered by *R*1. The bias from *R*3 is normally approximately 1.5 volts. The potentials *B*1, *B*2, and *C* may be supplied from batteries or well-regulated power supplies; *B*2 must be isolated from ground, so that the alpha-transparent electrode can always be maintained at ground potential. The tube heaters are supplied with 6.3 volts alternating current regulated by means of a Sola constant voltage transformer. The tubes may have to be selected for low grid current: Type 6AK5 or 6Q7 can be substituted for Type 6AG5.

The initial solution to alpha-transparent electrode potential is set by the adjustment of *R*3 and *R*4, and by the initial position of *R*1. The linear change in the voltage of the grid of V_2, as *R*1 is traversed, produces a linear change in the current through V_5. This changes the solution to alpha-transparent electrode potential, which change is impressed on the grid of V_1. Hence the difference amplifier, V_1 and V_2, remains balanced as long as the change in the solution to alpha-transparent electrode potential is linear. Any nonlinearity in the potential change of this electrode, due to polarization or amplifier nonlinearity, results in unbalance of the differential amplifier. This causes the current through V_5 to change in direction and magnitude to restore linearity. The corrective action is rapid, and the potential of the alpha-transparent electrode changes linearly with time.

An ionization chamber is mounted directly below the platinum foil (Figure 1). The ionization chamber consists of a cylinder, fabricated of platinum 0.005 inch thick, 0.32 inch in diameter, and 2.5 inches long. A platinum wire, 0.030 inch in diameter, rounded at one end, and located at the axis of the cylinder, is used as the positive electrode. The voltage developed across a 10^{11}-ohm resistor placed in series with the ionization current circuit is fed to the input of a vibrating reed electrometer (Applied Physics Corp.) and is continuously recorded by a Speedomax potentiometer connected to the electrometer output. If desired, both cell current and ionization current can be recorded simultaneously on a multipoint recording potentiometer. Cell currents can be measured by placing a resistor in the external current circuit and measuring the potential drop across the resistor.

PROCEDURE

After the cell containing polonium solution has been arranged as shown in Figure 1, the open-cell potential of the alpha-transparent electrode is compared manually with the potential of a reference electrode. The potential is made more negative by actuating the motor-driven potentiometer. The ionization current is continuously recorded on a strip chart and the potentials are manually recorded on the same chart when measured. As the potential sweep is practically linear with time, intermediate potentials between readings can be determined by interpolation. After an increase in ionization current is recorded, indicating a polonium deposition, the direction of potential sweep may be reversed. A decrease in the ionization current indicates dissolution of the electrode deposit.

IONIZATION CURRENT *vs.* CELL CURRENT

The dissimilarity in behavior of cell current and ionization current during cathodic polarization of an electrode in a polonium solution in hydrochloric acid is shown in Figure 3. Although the ionization current base line remained flat between potentials of 1.0 to 0.49 volt positive to the silver–silver chloride reference electrode, the cell current underwent a decided increase, starting at 0.8 volt. This current was probably associated with oxygen reduction. The final cell current inflection at −0.1 volt was associated with hydrogen reduction. An ionization current inflection, caused by the deposition of polonium, occurred at 0.49 volt. Additional ionization current inflections at potentials more negative than 0.49 volt were recorded in subsequent experiments.

SIGNIFICANCE OF IONIZATION CURRENT INFLECTIONS

A hypothetical ionization current record of the deposition of a radioelement having two oxidation states in solution is shown in Figure 4.

At point *A*, the electrode potential is made negative by an external controlling circuit until a deposition occurs at *B*. The direction of potential sweep is reversed at *C*, so that the potential

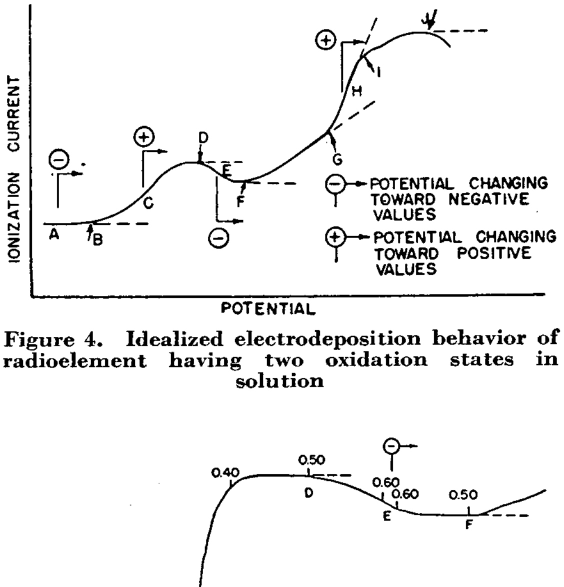

Figure 4. Idealized electrodeposition behavior of radioelement having two oxidation states in solution

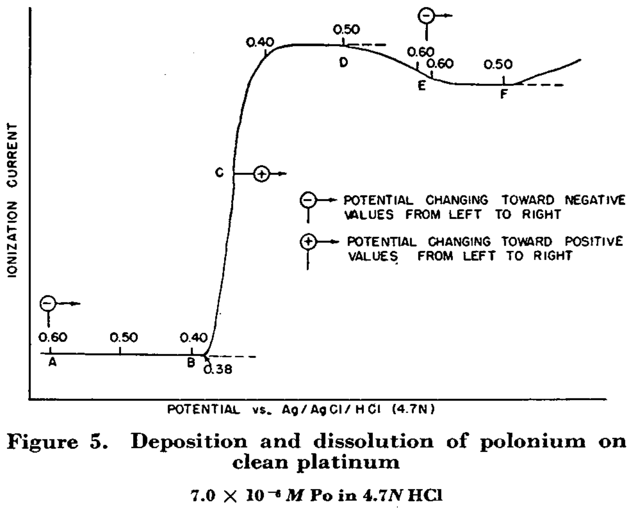

Figure 5. Deposition and dissolution of polonium on clean platinum

7.0×10^{-6} *M* Po in 4.7*N* HCl now becomes increasingly more positive. The radioelement starts dissolving from the electrode at point *D*. The potential sweep is reversed again at *E*, and a cathodic inflection is recorded at *F*. If the electrochemical system is reversible, potential *D* should agree with potential *F*. It would not necessarily be expected, however, that the initial deposition potential, *B*, should agree with *D* and *F*, since at *B* no equilibrium between the reduced form of the radioelement and its ions would have been established. If, after *F*, the potential is made increasingly more negative, a second inflection at *G* indicates that the radioelement has been reduced either from a second oxidation state or to a solid phase different from that of the first couple. The potential is reversed at *H*, and the inflection at *I* indicates that deposition no longer occurs from the second oxidation state, but the increase in the ionization current at potentials more positive than *H* indicates that deposition is still occurring. At *J*, the potential of the electrode has become more positive than the most positive couple, and dissolution occurs. If the potential couples are reversible and if negligible material has been removed from the solution, potentials *G* and *I* should be identical and potentials *D*, *F*, and *J* should be the same.

PERFORMANCE

Tracings of typical ionization current values, recorded during the polarization of a platinum foil electrode in $7 \times 10^{-6}M$ polonium-210 solution in $4.7N$ hydrochloric acid are shown in Figure 5.

Starting at *A*, the potential was made more negative until an ionization current inflection at *B* indicated the polonium was being deposited. This first deposition on fresh platinum was not reproducible. Anodic polarization was started at *C*, the curve reached a maximum, and the dissolution of polonium started at *D*. The direction of potential sweep was again reversed at *E*, and a cathodic inflection was recorded at the same potential, *F*, as that of the previous anodic inflection, 0.50 volt.

Polonium deposition or dissolution inflections could be reproduced any number of times by fixing the electrode potential more negative or more positive than 0.50 volt. When the potential was allowed to become more negative than 0.27 volt, a second cathodic inflection was recorded, suggesting a second oxidation state of polonium. The anodic inflection at 0.27 volt could not be resolved, but the anodic inflection at 0.50 volt still remained. When the potential was made 0.70 volt positive to the reference electrode and then decreased, a third cathodic inflection at 0.56 volt was recorded.

DISCUSSION

The nonreproducibility of the potential of the initial polonium deposit on fresh platinum may be explained by alloy formation between the metals. The shift of subsequent deposition potentials to more positive values than that of the initial deposit is similar to the behavior of silver deposition from very dilute solutions reported by Rogers, Krause, Griess, and Ehrlinger, (*3*). These authors found that for concentrations of silver insufficient to cover a platinum electrode ($10^{-7}M$) the resulting deposition curves often shifted to a more "noble" potential than that predicted by the Nernst equation.

Inflections at 0.56 volt were noted only after anodizing the platinum electrode, so this potential was probably associated with platinum reduction and the carrying of the chemically similar element, polonium. Anodic dissolution never completely removed polonium from platinum electrodes.

ACKNOWLEDGMENT

The authors wish to thank P. E. Ohmart and W. H. Baker for assistance with the instrumentation.

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RECEIVED for review July 21, 1955. Accepted December 8, 1955. Abstracted from U. S. Atomic Energy Commission Report MLM-909, October 28, 1953. Mound Laboratory is operated by Monsanto Chemical Co. for the U. S. Atomic Energy Commission under Contract AT-33-1-GEN-53.

Analysis of Phosphorus Compounds

Rapid Hydrolysis of Condensed Phosphates in Volumetric Analyses

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The step that requires the most time in the analysis of phosphates by pH titration is hydrolysis of the condensed phosphates to orthophosphates. The time required for hydrolysis has been reduced from a minimum of 8 hours to less than 1 hour by boiling the sample to dryness from strongly acidic solutions. Alkali metal chlorides are added to the acidic solutions to prevent recondensation of the orthophosphate when heated to dryness. No loss in the quality of the results is caused by the more rapid method.

THE analysis involved in a quantitative determination of the various species of condensed phosphates in complex mixtures invariably requires a hydrolysis step. In colorimetric analysis (*2*) the hydrolysis step is relatively rapid, but the volumetric analysis of condensed phosphates presents several problems not encountered in the other methods. If a large excess of a volatile acid is used to hasten the hydrolysis of the phosphates, this acid must be removed or neutralized before the phosphate may be titrated. The neutralization of the excess acid with concentrated caustic is not recommended because of the danger of carbonate contamination and the ultimate interference in the analysis. Carbonate not only titrates as a weak acid but also causes unstable end points.

A difficulty involved in removing the volatile acid by boiling it away occurs during the final stages of the evaporation and appears to be caused by a small amount of recondensation of the orthophosphates as the solution boils to dryness. This difficulty may be avoided by adding sufficient sodium chloride or potassium chloride to the sample to be analyzed to furnish an excess of alkali metal ions at the completion of the reaction. When potassium chloride is present, the orthophosphates do not recombine to form condensed phosphates, but instead the hydrogen of the orthophosphate interacts with the chloride ions to form volatile hydrochloric acid which is boiled away. The residue of this reaction is a mixture of orthophosphate and any excess of sodium or potassium chloride which was originally added to the solution. The orthophosphate is slightly more basic than would have resulted from the hydrolysis if the strong electrolyte had not been added.

Table I. Titration of Pure Phosphates after Hydrolyzing

Phosphate	Calculated Weak Acid to Be Titrated, Meq. Theory	Weak Acid Found, Meq.[a]	Deviation from Theory, Part/100
$Na_5P_3O_{10}$	4.531	4.540	+0.2
	3.873	3.897	+0.6
	4.531	4.544	+0.3
	3.874	3.900	+0.6
	3.581	3.548	−0.9
$Na_4P_2O_7$	3.677	3.701	+0.6
	3.610	3.615	+0.1
$[KPO_3]n$	4.395	4.383	−0.3
	4.356	4.401	+1.0
$Na_4P_4O_{12}$	4.402	4.386	−0.4
	4.377	4.337	−0.9
$Na_3P_3O_9$	5.250	5.271	+0.4
	5.250	5.233	−0.4
		Absolute average	−0.5

[a] Determined with Precision-Dow automatic Titrometer.

EXPERIMENTAL

Use an analytical balance to weigh about 0.5 gram of the mixture of condensed phosphates to be analyzed. Transfer the sample to a 250-ml. beaker, and add 50 ml. of water. Next, add about 10 ml. of concentrated hydrochloric acid to the solution of phosphates, and then add ca. 1 gram of potassium chloride (or sodium chloride). Potassium orthophosphates are more soluble than sodium orthophosphates, and less splattering is encountered as the sample goes to dryness when potassium chloride is used in preference to sodium chloride. Place a watch glass over the beaker, and boil the sample to dryness. Take care to reduce the temperatures of the sample as the crystals begin to form; otherwise, the sample will splatter. Next, dilute the sample to 50 ml. and titrate the sample as orthophosphate, using the procedure recommended in the previous paper (*3*).

DISCUSSION OF RESULTS

Table I is a compilation of some typical results obtained by the rapid method. The results are as good as those obtained by the slower method, and less than one tenth of the time was required to obtain the results. Most soluble phosphate may be analyzed for percentage of phosphorus pentoxide by the above method, but the procedure is specifically designed for alkali metal phosphates. Any anionic substance exhibiting a weak acid titration will interfere with the above analysis, as will amphoteric cations such as aluminum. In case of interference of this type, it is recommended that one of the molybdate methods be employed to analyze the samples after they have been hydrolyzed (*1*).

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RECEIVED for review November 4, 1955. Accepted December 9, 1955.

Total Naphthenes in Gasoline by Refractivity Intercept

Analysis of Six- to Eight-Carbon Saturates

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Refractivity intercept has been used for many years to determine naphthenes in gasoline. The method is applicable to mixtures of paraffins and monocyclic naphthenes; the presence of polycyclic naphthenes causes erroneous results. Improved graphs, one for the C_6 to C_8 range and one for the C_6 range only, are presented and evaluated. The graphs are based on the relative amounts of individual saturated hydrocarbons in crudes. When applied to the C_6 to C_8 range of straight-run gasoline, total naphthenes can be determined with an accuracy of ±2%. Application to other gasolines is dependent upon the type of paraffins present.

THE yield of benzene, toluene, and xylenes produced by catalytic reforming of straight-run naphtha depends to a large extent on the amount of naphthenes (cycloparaffins) in the charge stock. A comparison of various methods for determining total and individual naphthenes was recently reported (*20*). In one of those methods the total naphthenes in the paraffin-naphthene (saturated) fraction were determined from refractivity intercept–density correlations requiring only the experimental determination of density and refractive index. Refractivity intercept is the refractive index minus one half the density ($n - d/2$) (*18*).

It has been known for a number of years that refractivity intercept could be used for naphthene determination (*2, 11, 13, 16–18, 26*). When the first refractivity intercept graph (*2*) was derived for naphthene determination, very little information was available on the relative amounts of individual hydrocarbons in gasolines. Since then, American Petroleum Institute Project 6 (*19, 23*) and the Bureau of Mines, Petroleum Experimental Station (*24*), have determined individual hydrocarbons in about 35 different crudes. A study of the hydrocarbon distribution in several crudes (*9, 19, 23–25*) led to the development of new refractivity intercept–density graphs. This paper presents improved graphs for the C_6 to C_8 range. The improvement has

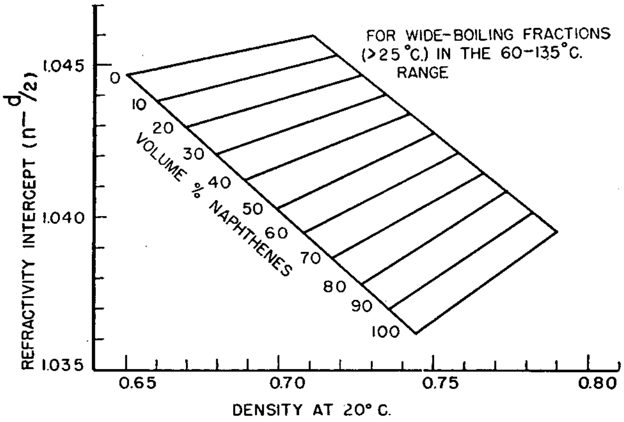

Figure 1. Total naphthenes

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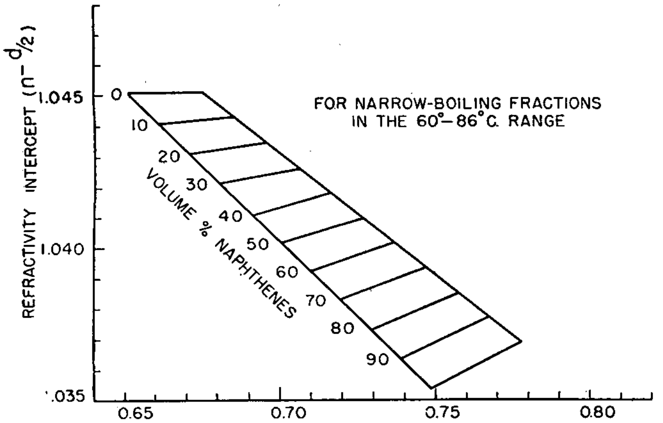

Figure 2. Six-carbon naphthenes

Table I. Data for Construction of Figure 1

Naphthenes, Volume %	d_4^{20}	$n - d/2$	d_4^{20}	$n - d/2$
0	0.650	1.04470	0.710	1.04600
10	0.660	1.04380	0.715	1.04528
20	0.671	1.04300	0.725	1.04466
30	0.680	1.04210	0.730	1.04390
40	0.690	1.04128	0.740	1.04334
50	0.700	1.04040	0.750	1.04280
60	0.710	1.03962	0.755	1.04200
70	0.720	1.03884	0.765	1.04142
80	0.730	1.03806	0.770	1.04064
90	0.740	1.03730	0.780	1.04008
100	0.745	1.03620	0.790	1.03950
Boundary lines	0.650 0.710	1.04470 1.04600	0.745 0.790	1.03620 1.03950

Table II. Data for Construction of Figure 2

Naphthenes, Volume %	d_4^{20}	$n - d/2$	d_4^{20}	$n - d/2$
0	0.652	1.04505	0.675	1.04515
10	0.663	1.0441	0.685	1.0443
20	0.671	1.0431	0.690	1.0434
30	0.683	1.0422	0.704	1.0426
40	0.691	1.0412	0.715	1.0418
50	0.700	1.0402	0.726	1.0410
60	0.711	1.0393	0.734	1.0401
70	0.722	1.0384	0.745	1.0393
80	0.731	1.0374	0.757	1.0385
90	0.740	1.0364	0.765	1.0376
100	0.748	1.0354	0.776	1.0368
Boundary lines	0.650 0.670	1.0452 1.0455	0.750 0.780	1.0352 1.0368

been achieved by adjusting the paraffin and naphthene lines for the approximate distribution of the individual hydrocarbons in naturally occurring petroleum.

Use of this refractivity intercept method for naphthenes is limited to samples in which polycyclic naphthenes are negligible. Polycyclic naphthenes have a relatively low refractivity intercept and a high density, which cause them to have an exaltation effect on the total naphthene content determined by this method. In many crudes, this limits the application of the method to fractions boiling below 150° C. For this reason and because individual hydrocarbon data on crudes are not available in higher boiling ranges, the reliability of the method can only be stated in the C_6 to C_8 range, where it is accurate to ±2%.

NEW REFRACTIVITY INTERCEPT GRAPHS

The graph for the C_6 to C_8 range, for wide-boiling saturated hydrocarbon fractions in the 60° to 135° C. (140° to 275° F.) range, is shown in Figure 1. On samples containing negligible amounts of polycyclic naphthenes, this graph may be used for higher-boiling ranges by extending the lines to the right.

Special graphs are necessary for accurate naphthene analysis of narrow-boiling fractions (*13*). For example, Figure 2 was specially drawn for determining the C_6 naphthenes, methylcyclopentane and cyclohexane. This graph may be used for any saturate fraction in the 60° to 86° C. (140° to 187° F.) range.

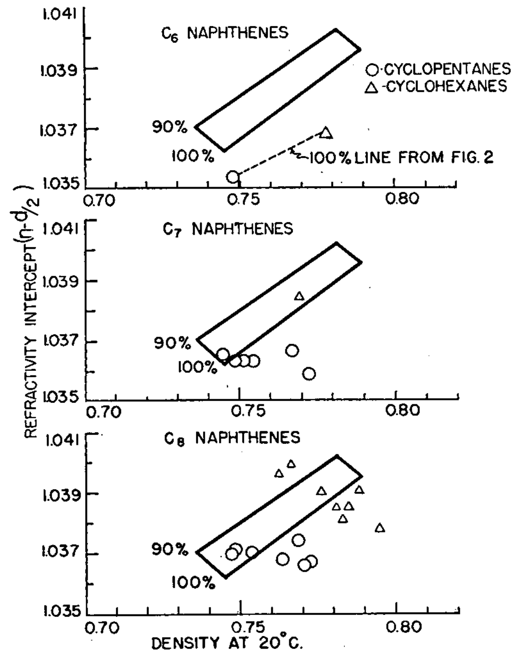

Figure 3. Placement of 100% naphthene line

Tables I and II give data for construction of the two refractivity intercept–density graphs. The graphs should be drawn on coordinate paper so they can be read to 0.0001 unit in refractivity intercept and 0.001 unit in density.

PARAFFIN AND NAPHTHENE DISTRIBUTION

The new graphs are based on the distribution and amount of individual saturated hydrocarbons in the C_6 to C_8 range of crudes. The 100% naphthene lines are based on the relative amounts of individual naphthenes, and the 0% naphthene lines are based on the relative amounts of individual paraffins.

Individual naphthene isomers are plotted in Figure 3 and compared with the position of the 100% naphthene line from the general purpose graph. Crudes differ considerably in the relative amounts of naphthenes of the cyclopentane and cyclohexane types. However, within the two classes the relative amounts of the individual isomers remain fairly constant (*19*). Fortunately, differences in the ratio of cyclopentanes to cyclohexanes do not affect the placement of the 100% naphthene line in the C_6 to C_8 range. As shown in Figure 3, the cyclopentanes fall at the lower end of the line and the cyclohexanes at the higher end.

The new 100% naphthene line differs from that on the previous published graph (*2, 17*) by an amount equivalent to only 1% naphthenes. This is a very minor change. The position of the C_6 naphthenes with relation to the general purpose graph (top of Figure 3) shows why a special graph is needed for the C_6 range. The 100% naphthene line in the special graph, Figure 2, connects these two points.

The relative amounts of paraffin isomers are much more important in deriving an accurate refractivity intercept chart. Individual isomers are plotted in Figure 4. The new 0% naphthene line differs considerably from the previously published graph because of the predominance of normal and singly branched paraffins in petroleum fractions. As shown at the bottom of Figure 4, the change amounts to about 4 to 7% in very low naphthene content samples.

Literature data (*19*, *23*, *24*) stress the predominance of normal and single methyl-branched paraffins in straight-run gasoline. Even in crudes that can be classified as high in isoparaffins, such as that from Winkler, Tex. (*19*, *23*), the amount of paraffins containing more than a single methyl-branch is only about 10% of the total paraffin content in the C_6 and C_7 range. Doubly branched paraffins are present in small amounts and triply branched paraffins are absent or barely detectable (*9*, *24*, *25*).

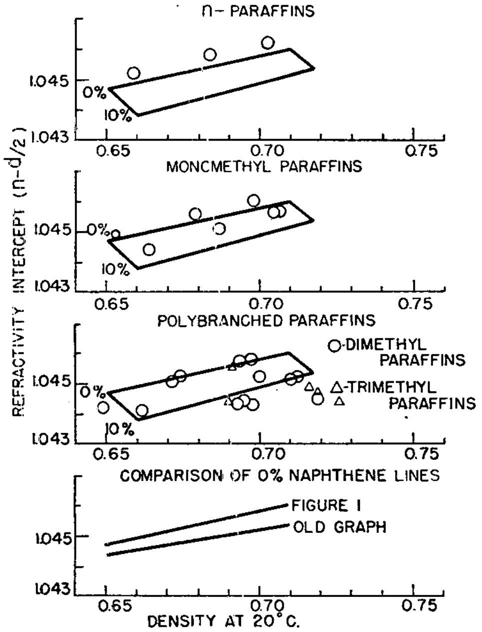

Figure 4. Placement of 0% naphthene line

Literature data on the products from thermal and catalytic cracking show a difference in the paraffin isomer distributions. Thermal gasolines (*8*, *10*) contain very few highly branched paraffins; the normal paraffins and single methyl-branched paraffins are in about the same relative amounts as found in virgin naphthas. Catalytic gasolines (*8*, *10*, *12*, *14*, *21*, *23*) contain relatively low amounts (<15%) of normal paraffins, large amounts (50 to 75%) of single methyl-branched paraffins and 15 to 30% of multibranched paraffins.

Practically no data have been published on the paraffins in catalytic reformates from petroleum fractions. Data obtained in this laboratory (*25*) show the following relative amounts of paraffins in several different reformates.

		Normal Paraffins, %	Branched Paraffins, %	
			Monomethyl	Multibranched
C_6 and C_7 Reformates from 65° to 104° C. (150° to 220° F.) naphtha				
A	C_6	63	35	2
	C_7	37	48	15
B	C_6	42	51	7
	C_7	25	52	23
C_8 Reformate from 104° to 146° C. (220° to 295° F.) naphtha				
	C_8	9	55	36

The 0% naphthene line in the new chart has been derived for the paraffin isomer distribution in straight-run gasolines; it is probably just as accurate for thermal cracked gasolines. It will be somewhat less accurate for catalytic reformates and catalytic cracked gasolines which contain larger amounts of doubly branched paraffins. The graphs should not be used for mixtures containing high concentrations of doubly and triply branched paraffins, such as alkylate.

The graphs shown in Figures 1 and 2 were derived from physical property data for individual hydrocarbons (*1*). The properties were weighted according to the concentration of the hydrocarbons in the boiling range of interest. Composition data from the infrared analysis of a series of narrow-boiling fractions were available on a blend of Gulf Coast Crudes (*25*). Figure 1 was drawn using data for all the individual C_6 to C_8 saturated hydrocarbons present. Figure 2 was drawn using data for the C_6 naphthenes and the paraffins boiling in the same range. Similar graphs have been constructed in like manner to cover other narrow-boiling ranges for special naphthene analysis problems.

TESTING NEW GRAPHS

The 0 and 100% naphthene lines on the general purpose graph, Figure 1, were checked using published data for individual C_6 to C_8 saturated hydrocarbons in Ponca, Okla., and East Texas crudes (*9*, *19*). The deviations of the individual paraffins from the 0% naphthene line and the deviations of the individual naphthenes from the 100% naphthene line were determined and weighted deviations calculated from the percentage of the individual compounds. Weighted deviations were calculated for both Figure 1 and the previous graph (*17*). The comparison in Table III shows that the new graph represents the paraffins in virgin naphtha better than the previous graph, but for naphthenes it represents only a slight improvement. The new graph is just as accurate for the hydrocarbon distribution in Ponca and East Texas crudes as for the Gulf Coast crude used in the derivation.

Table III. Comparison of New Graph to Old Graph for Hydrocarbon Distribution in C_6 to C_8 Range of Three Crudes

Crude	Paraffins		Naphthenes	
	Figure 1	Old graph (*17*)	Figure 1	Old graph (*17*)
	Weighted Average Deviation[a], %			
Ponca, Okla.	4.4	7.7	6.0	6.4
East Texas	4.2	6.8	6.1	6.7
Gulf Coast	4.4	7.0	6.5	7.0
	Weighted Deviation of Average[b], %			
Ponca, Okla.	−1.3	−4.3	−0.4	+0.6
East Texas	−0.7	−5.5	+0.4	+0.3
Gulf Coast	−0.7	−5.8	−0.7	−0.7

[a] Average deviation is average of all values without regard to sign.

[b] Deviation of average is average of all values including plus or minus signs.

Weighting based on the following equations:

$$\text{Weighted deviation for paraffins} = \frac{\Sigma\ (\text{dev. from } 0\% \text{ line})\ \%\ \text{paraffin in crude}}{\Sigma\%\ \text{paraffin in crude}}$$

$$\text{Weighted deviation for naphthenes} = \frac{\Sigma\ (\text{dev. from } 100\% \text{ line})\ \%\ \text{naphthene in crude}}{\Sigma\%\ \text{naphthene in crude}}$$

For petroleum fractions, naphthene determinations by the refractivity intercept graphs, Figures 1 and 2, have been compared to results obtained by infrared spectrometry and by carbon-hydrogen analysis. Data are shown in Tables IV and V. A brief description of the experimental methods follows.

Dearomatization. Saturate fractions were prepared by acid treating straight-run naphtha or reformates at 0° to 5° C. A 10-ml. fraction was treated as described in (*6*) or 100 to 300 ml. of sample was treated using larger scale apparatus. The raffinate was neutralized and washed with water. It has been shown (*22*) that treating with sulfuric acid–phosphorus pentoxide completely removes aromatics and olefins and leaves a saturate fraction satisfactory for further analysis. Silica gel adsorption (*3*) has also been used for dearomatization.

Physical Properties. Densities were determined using 5-ml.

Table IV. Total Naphthenes in C_6 to C_8 Saturates

[Comparison of refractivity intercept method (Figure 1) with infrared spectrometry]

Source of Saturate Fractions	Boiling Range, ° C.	Physical Properties of Saturates n_D^{20}	d_4^{20}	$n - d/2$	Volume % Naphthenes $n - d/2$	Infrared[a]	Difference, $(n - d/2) - IR$
Straight-run gasoline							
1	60–105	1.4051	0.7289	1.0406	60	57	+3
2	60–110	1.4040	0.7255	1.0413	52	52	0
3	60–110	1.4043	0.7265	1.0410	55	53	+2
4	60–110	1.4043	0.7268	1.0409	56	55	+1
5	60–110	1.4035	0.7245	1.0413	52	52	0
6.	100–140	1.4117	0.7384	1.0425	47	45[b]	+2
Reformate							
1	60–110	1.3846	0.6806	1.0443	10	5	+5
2	60–110	1.3875	0.6866	1.0442	13	10	+3

[a] Sum of all individual naphthenes by analysis of narrow-boiling fractions.

[b] Includes sum of individual C_7 and C_8 naphthenes, 35%, plus 10% unidentified naphthenes determined by refractivity intercept on the C_9 fraction.

Table V. Determination of C_6 Naphthenes

[Comparison of refractivity intercept method (Figure 2) with infrared and lamp hydrogen methods]

Sample	Tests on Saturates n_D^{20}	d_4^{20}	$n - d/2$	Lamp H g. H_2O/g.	Volume % Napthenes I $n - d/2$	II Infra-red[a]	III Lamp H[b],	Difference I–II	I–III
Straight-run gasolines (60° to 81° C.)									
1	1.4013	0.7221	1.0402	1.3520	56	56	57	0	−1
2	1.4015	0.7229	1.0401	1.3482	57	56	59	+1	−2
3	1.4007	0.7209	1.0403	1.3535	55	57	56	−2	−1
4	1.4006	0.7209	1.0402	1.3486	56	55	59	+1	−3
5	1.3992	0.7186	1.0399	1.3507	57	...	58	...	−1
6	1.3967	0.7119	1.0408	1.3628	48	...	51	...	−3
7	1.3991	0.7164	1.0409	1.3625.	48	...	51	...	−3
8	1.3968	0.7131	1.0402	1.3596	53	...	53	...	0
9	1.3957	0.7099	1.0408	1.3653	47	...	49	...	−2
Reformate (60° to 81° C.)	1.3836	0.6814	1.0429	1.4195	23	23	20	0	+3

[a] Sum of methylcyclopentane and cyclohexane.

[b] Calculated assuming a C_6 to C_7 paraffin ratio of 8 to 2.

pycnometers (*4*). Refractive indices were run on the Bausch & Lomb refractometer (*7*) or, in some cases, on the Valentine Abbe-type precision refractometer. The accuracy of both physical properties was within about ±0.0001, making the repeatability of the naphthene analysis by refractivity intercept at least ±2%.

Infrared. The individual hydrocarbons in narrow-boiling distillate fractions were determined using a Perkin-Elmer Model 12-A infrared spectrometer. A base line absorbance method similar to that described by Bell (*9*) was employed. Calibrations were established by using API standard samples of hydrocarbons. The analytical results are believed to be accurate to ±2% on individual fractions.

Lamp Per Cent Hydrogen. Saturate fractions were analyzed for per cent hydrogen. Naphthene values were calculated by the method of Hindin and Grosse (*15*). The ASTM lamp method (*5*) was modified to give an accuracy within ±0.04% for pure C_6 hydrocarbons. This makes the accuracy of the calculated naphthene values ±2%. The modifications to the lamp method consisted of icing the burner to reduce volatility of the light compounds and burning larger samples to reduce weighing errors.

Table IV compares total C_6 to C_8 naphthenes by refractivity intercept to the sum of individual naphthenes by infrared analysis of a series of distillate fractions. On five straight-run fractions where infrared was used to determine C_6 and C_7 naphthenes, the average difference between the methods is about 1%. On two reformates the agreement is not this good.

Table V compares total C_6 naphthenes by the special graph, Figure 2, to naphthenes by independent methods. The agreement between naphthenes by refractivity intercept and infrared on four straight-run fractions and the reformate fraction is within 1%. The average difference between naphthenes by refractivity intercept and lamp hydrogen is about 2% on the 10 fractions. Use of the special graph for narrow-boiling fractions is important. The high naphthene samples (>50%) in Table V, if analyzed by Figure 1, would show naphthene contents as much as 5% too high.

The two graphs presented in this paper have been used to analyze the cooperative sample circulated by the Subcommittee on Determination of Naphthenic Hydrocarbons of the Committee on Analytical Research of the American Petroleum Institute (*20*). This sample, a 170° to 220° F. fraction from California crude, was analyzed for C_6 naphthenes by twelve different petroleum laboratories and for total naphthenes by seven different laboratories. Table VI compares the average values from the cooperating laboratories to the values obtained by the refractivity intercept graphs. Six-carbon naphthenes were determined on the dearomatized 60° to 86° C. (140° to 187° F.) fraction separated by distillation. Total naphthenes were determined on the whole dearomatized sample. In each case the agreement is excellent.

USE OF REFRACTIVITY INTERCEPT GRAPHS

The refractivity intercept method can be conveniently used for routine naphthene analysis. Detailed infrared analysis is too time consuming to be used on a routine basis. The main advantage of the refractivity intercept method compared to the mass spectrometer is in the low cost of equipment and saving of calculation time. Refractivity intercept can be used in any laboratory where a dearomatization can be carried out and refractive index and density can be run on the saturates with the required precision. Because a difference of 1×10^{-4} in refractive index and 2×10^{-4} in density causes 1 to 2% difference in naphthene contents, these two properties must be precisely measured to obtain highest accuracy. Standardized methods are available for making these measurements (*4, 7*). Samples to be analyzed by this method should be debutanized and preferably depentanized if reliable results are to be obtained.

Table VI. Naphthene Analysis of API Cooperative Sample

Analysis	Properties of Saturates n_D^{20}	d_4^{20}	$n - d/2$	Volume % Naphthenes on Whole Sample: Refractivity intercept graph	Av. of cooperating laboratories	Diff.
Total C_6 naphthenes[a]	1.3962	0.7113	1.0406	17.1 (Fig. 2)	16.5[b]	+0.6
Total naphthenes	1.4025	0.7231	1.0409	51 (Fig. 1)	52[c]	−1

[a] Determined on 60–86° C. fraction.

[b] Average of methylcyclopentane plus cyclohexane values from 12 laboratories (*20*).

[c] Average of sum of individual or groups of naphthenes from 7 laboratories (*20*).

The new average graph is intended primarily for the analysis of straight-run gasoline or fractions having a boiling range greater than 25° C. (45° F.). It can be used to determine total naphthenes in the C_6 to C_8 range with an accuracy within 2%. It can be used with about the same accuracy on some higher boiling fractions by extending the lines to the right. The method should not be used for any fraction boiling higher than 200° C. (390° F.) nor for gasolines boiling higher than 150° C. (300° F.) and showing more than 60% naphthenes in the saturates. In these cases, inaccuracies due to the presence of dicyclic naphthenes will result in high values. The general graph can be used on the saturates from thermal cracked gasolines, but will probably show 1 to 3% too much naphthenes in saturates from catalytic reformates and catalytic cracked gasolines. The graph should not be used for gasoline blends containing more than 10% alkylate or other highly branched paraffins.

To obtain an accuracy within 2%, special charts such as Figure 2 should be used for narrow-boiling fractions in the C_6 and C_7 range. There is little use in deriving special charts for the C_8 range and higher. Fractions in this range contain too many isomers unless they are prepared by extremely efficient fractionation.

ACKNOWLEDGMENT

The authors appreciate the helpful discussions with Stewart S. Kurtz, Jr. Infrared analyses were obtained under the direction of E. J. Rosenbaum and Anne R. Donnell.

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Received for review July 30, 1955. Accepted January 10, 1956.

Sealed-Tube Combustion Technique for Determination of Deuterium in Organic Compounds

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A new, more convenient, and more precise method for the determination of deuterium in combustible substances is described. Sample size has been reduced to from 5 to 10 mg. Deuterium concentrations in the range of 1 atom % may be determined with a precision of ±1%.

SINCE publication of the previous paper (*2*), some important changes have been introduced, improving both the reliability and convenience of the method for determining deuterium in combustible substances. The usefulness of the standard or dynamic combustion technique was limited by a memory effect of varying seriousness. When pronounced, this effect necessitated prolonged conditioning of the system prior to any determination. It was with the hope of minimizing this problem that attention was turned to the development of the sealed-tube or static combustion technique.

EXPERIMENTAL

Nine-millimeter Vycor tubing (Corning) is cut into a 6-inch section and sealed at one end. The sample of deuterated material, sufficient to give 3 to 5 mg. of water on combustion, is introduced, followed by approximately 250 mg. of copper oxide prepared by oxidation of electrolytic grade copper powder [obtained from the United States Metals Refining Co. (*1*)]. To facilitate subsequent seal off, a capillary constriction is drawn close to the top. The combustion tube is then attached to a pumping system (*1*) and the sample is evacuated for 2 hours at 105° to 110° C. The tube is then sealed at the constriction and placed in a furnace at 750° to 800° C. for 30 to 40 minutes. Care should be taken not to exceed the maximum temperature, since above 800° C. these ampoules tend to burst. Following combustion, the ampoule is enclosed in a protective cylindrical wire screen, inserted into the ampoule breaker (*1*), which is in turn inserted into the water distillation train (Figure 1). With stopcocks 2, 4, and 7 closed, the apparatus is pumped out for 5 minutes. Stopcock 8 is closed, and a 10-mg. dose of water of appropriate deuterium content is introduced into the system from the water reservoir by means of a dosing stopcock (Machlett). By appropriate deuterium content is meant water within 0.3 atom % deuterium of that expected for the sample. After 5 minutes have been allowed for isotope equilibration, pumping is resumed for 5 minutes. The system is isolated by closing stopcock 8, and tested for vacuum tightness by the vacuum thermocouple gage for 5 minutes. It is advisable to isolate the gage whenever water is in the system. The desensitization of the gage which occurs on contact with water can be corrected only by continuous pumping for several hours. If the system is vacuum tight, stopcocks 1, 3, and 5 are closed, and the ampoule is broken by turning the knob of the breaking device. The U-trap is immersed 3 to 4 inches in a dry ice–acetone freezing bath, stopcocks 3, 5, and 8 are opened in that order, and the system is pumped out for 1 minute. Stopcock 8 is closed and the freezing bath is transferred to the

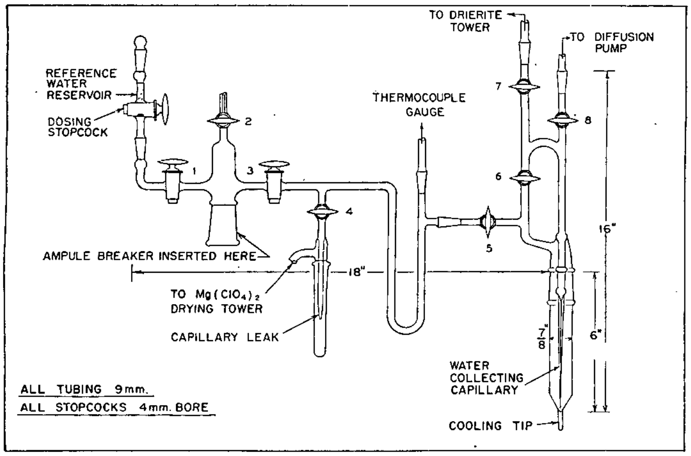

Figure 1. Diagram of sealed-tube combustion apparatus

cooling tip of the jacket surrounding the capillary. After 5 minutes have been allowed for distillation, the freezing bath is removed, and the system is brought to atmospheric pressure by opening the dry air inlet (stopcock 4). Stopcock 7 is opened, stopcock 6 is closed, and the water is drawn into the capillary by gentle suction applied through the Drierite tower. The jacket is removed, and the microcell is slowly raised by a screw and platform device until the capillary tip penetrates about $^1/_8$ inch into the entrance port of the cell. The application of gentle pressure through the Drierite tower forces the water into the cell, which fills automatically by capillary action.

The infrared spectrophotometry operation is the same as described in the previous papers (*2*, *3*), except that a metallized glass reference state has been substituted for the wire screen. The use of a wire screen had a number of undesirable features (*2*) which induced a search for a more ideal reference state. The substitution of a semitransparent metallized glass plate avoided these difficulties without sacrificing the advantages of the wire screen over the more conventional reference states. Thermostating of the microcell is accomplished by circulating water from a typical laboratory thermostat through an annular ring built into the cover of the spectrometer and which fit snugly around the cell adapter.

Table I. Memory Effect

Dose No.	Deuterium Found, %
1	1.78
2	1.94
3	1.97
4	1.99
5	1.98
6	1.98

DISCUSSION

One of the strongest incentives for modifying the previous technique was the need for analyzing smaller samples. To this end, a typical Perkin-Elmer microcell (Model 099–0018) was modified by removing the metal capillary filling tube and taper reaming the exposed cell entrance port slightly to accommodate the tip of the water-collecting capillary. Penetration of the capillary well within the port makes for more efficient water transfer by minimizing dead volume losses. In this manner, minimal liquid water requirements were lowered to 1 to 2 mg. This accomplishment had limited value with the dynamic combustion technique, as the use of smaller samples was precluded by the excessively large memory effect. In developing the subject procedure, no acidic combustion products were observed to form from nitrogen or sulfur-containing compounds, as was experienced with the previous dynamic technique. This permitted elimination of the barium carbonate from the apparatus, a most fortunate occurrence, since subsequent experiments proved this material to be mainly responsible for the memory effect. With the present setup, one dosing will equilibrate the system at any deuterium level between 0 and 2.2% deuterium. Where the ultimate in accuracy is desired, two dosings are made.

To illustrate the magnitude of the memory effect in the present procedure, the following experiment was performed. The system was initially flushed out with ordinary water until the exuent and reservoir waters gave identical absorbances. Then from a water reservior containing 1.98% deuterium, 7-mg. dosings were passed through the system and the per cent deuterium was determined. Pump out time between runs, and conditioning time for the doses were both standardized at 10 minutes.

The data in Table I confirm the previous statement that one dosing is generally sufficient to neutralize memory effects. Even without dosing, the maximum error for any determination is no greater than 10%. On the basis of these observations, one can account for the memory effect in terms of approximately 0.5 mg. of adsorbed but exchangeable water on the interior surfaces of the apparatus. It follows, then, that when samples are within 0.3% deuterium of the equilibrated system level, memory perturbations become subdetectable.

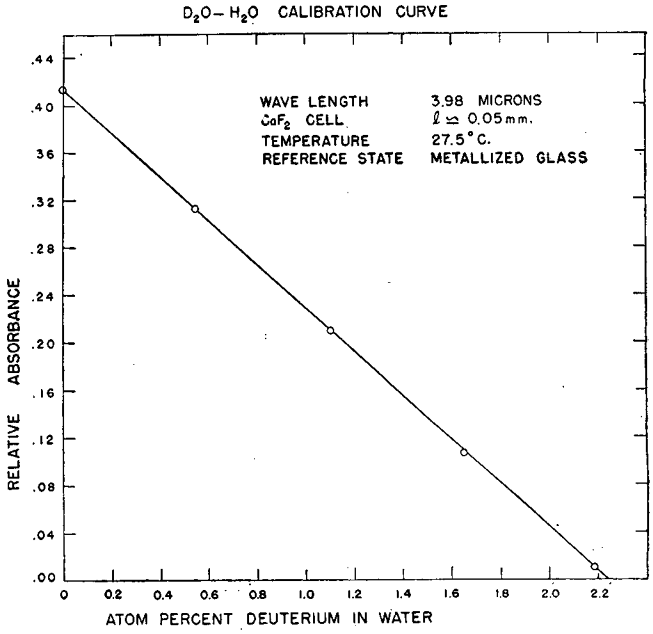

Figure 2. Deuterium oxide–water calibration curve

All the deuterium oxide samples used in establishing the calibration (Figure 2) were derived from dilutions of a master standard prepared in turn by a dilution of 99.8% deuterium oxide (obtained from Stuart Oxygen Co. by license from the Atomic Energy Commission). The dilutions were carried out on a weight basis with doubly distilled water. All points were averages of at least ten determinations made over a 5-week period. The precision was constant over the entire range, the average deviation being 1%. The curvature observed previously at higher deuterium levels (*2*) is undetectable up to 2.2% deuterium.

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Received for review October 8, 1955. Accepted January 25, 1956.

Carbazole Estimation of Hexoses in Connective Tissue Extracts and Their Hydrolyzates

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A modification of the carbazole–sulfuric acid method was used to show that fucose, galactose, glucose, and mannose are not significantly decomposed by heating with the hydrogen form of a cation exchange resin for 96 hours. Application to the analysis of connective tissue extracts which contained fucose, galactose, glucose, and mannose, and to their hydrolyzates, showed that hexoses could be accurately determined without knowledge of the exact ratios in which they occurred.

THE carbazole–sulfuric acid technique for the estimation of hexoses (*1, 2, 7, 8*) was modified by the introduction of a new system of blanks (*11*) and used to determine the polysaccharides of serum (*11*) and influenza virus (*10*). Holzman, MacAllister, and Niemann (*9*) subsequently made a systematic study of the variables influencing the determination and established optimum conditions for the reaction by adjusting the concentrations of sulfuric acid and carbazole, the period of heating, and the volumes of solutions to be used. In the present investigation a modified procedure was developed on the basis of previous findings (*9, 11*) and used to estimate the carbohydrate content of connective tissue extracts (*4, 6*), the degree of decomposition caused by heating monosaccharides with a cation exchange resin (*3*), and carbohydrate-protein complexes with the resin, hydrochloric acid, and sulfuric acid.

EXPERIMENTAL

Carbazole–Sulfuric Acid Technique. REAGENTS. Reagent grade carbazole was purified by dissolving it in 84% sulfuric acid, precipitating with water, and recrystallizing the product from alcohol and then from toluene. The stock solution made up to contain 0.5% (w./v.) of the purified carbazole in absolute alcohol should remain colorless when mixed with 84% sulfuric acid. (A comparable product was obtained after four sublimations.)

Sulfuric acid was of reagent grade, diluted to 84% by weight (30.3*N*). The concentration was checked by titration with sodium hydroxide.

SUGARS. With the exception of glucose, which was a standard sample obtained from the National Bureau of Standards, the sugars were commercial samples which had been recrystallized. Their purity was checked by polarimetric measurements.

MODIFIED PROCEDURE. Two reagents were prepared. For Reagent A 8.35 ml. of absolute alcohol was made up to 250 ml. with 84% sulfuric acid. For Reagent B 8.35 ml. of the 0.5% solution of carbazole in absolute alcohol was made up to 250 ml. with 84% sulfuric acid.

Reagents A and B were stored at 4° C. for as long as 1 week, and allowed to warm up to room temperature just before being used.

For any given determination, three blank solutions were used: the "over-all reference blank" containing 9 ml. of Reagent A and 1 ml. of water; the "sugar (or unknown) blank" consisting of 9 ml. of reagent A and 1 ml. of the solution under investigation; and the "carbazole blank" containing 9 ml. of Reagent B and 1 ml. of water. The final solution for the measurement of color development between carbazole–sulfuric acid and carbohydrate was made up from 9 ml. of Reagent B and 1 ml. of the sugar (or unknown) solution.

The 9-ml. aliquots of Reagents A and B were placed in 8-inch borosilicate glass test tubes, and 1-ml. portions of water and sugar (or unknown) solution were carefully layered above them. The contents of the tubes were then thoroughly mixed by shaking and the mixture was heated in a boiling water bath for 13 minutes. The tubes were removed and cooled in an ice-water mixture for 10 minutes. (If necessary, they could then be allowed to stand at room temperature for 3 hours without change in the spectrophotometric readings.)

Absorbances were measured on these solutions using a Beckman DU spectrophotometer. The instrument was adjusted to zero with the over-all reference blank and the absorbances of the other three solutions were measured against this blank. In order to obtain the corrected absorbance, the sum of the readings for the sugar (or unknown) blank and the carbazole blank was subtracted from the reading for the standard sugar (or unknown) solution (*11*).

Plots were made of absorbance against wave length over the range 400 to 580 mμ to obtain absorption curves for the simple sugars glucose, galactose, mannose, glucuronic acid, and fucose, as well as mixtures of galactose-mannose (1:1), galactose-glucose-mannose (1:1:1), and galactose-mannose-fucose (1:1:1). These monosaccharides and mixtures showed maximum absorption at 548 mμ, except that fucose absorbed maximally at 528 mμ and glucuronic acid at 540 mμ.

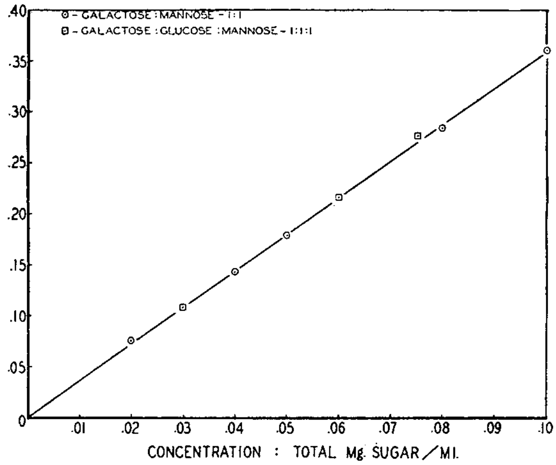

Figure 1. Relation of corrected absorbances (carbazole) to concentrations of galactose-mannose and galactose-glucose-mannose mixtures

Absorbances were measured at 548 mμ for galactose-mannose (1:1) over the concentration range of 0.02 to 0.1 mg. per ml. and for galactose-glucose-mannose (1:1:1) over the range of 0.03 to 0.075 mg. per ml. to obtain standard curves (Figure 1).

Recovery of Monosaccharides after Heating with Resin. RESIN. The resin, Permutit Q, a polystyrene sulfonic acid type of cation exchange resin, was acid-regenerated by shaking with 4.4*N* hydrochloric acid (900 ml. per liter of resin) in a separatory funnel, and then washed with large volumes of distilled water until the washings were free of chloride ions. The resin was refluxed for 7 days with several changes of distilled water until the washings, which were at first dark brown, became almost colorless, and was then air-dried. The material extracted from the resin with hot water absorbed strongly at 548 mμ and without this treatment there would have been a high blank in the following work, which involved heating solutions of monosaccharides and other substances with the resin prior to quantitative determination by the carbazole–sulfuric acid technique.

To determine whether the boiled resin was still effective as a catalyst for hydrolysis, several glycoproteins were hydrolyzed under the usual conditions (*4–6*) and the hydrolyzates were

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Table I. Corrected Absorbances (Carbazole Technique) of Sugar Solutions before and after Treatment with Permutit Q at 100° C.

Substances	Sugar Solution Concn., Mg./Ml.	Corrected Absorbances		
		Un-treated	Resin Treated	
			48 hours	96 hours
Fucose	0.20	0.150	0.147	0.148
Glucose	0.04	0.199	0.196	0.197
Cellobiose	0.04	0.193	0.190	0.186
Galactose-mannose (1:1)	0.04	0.141	0.136	0.139
Glucuronic acid	0.04	0.127	0.045	0.018

analyzed by paper chromatography. The results were the same as those obtained previously (*4–6*), the monosaccharides being detected after spraying with aniline hydrogen oxalate.

In order to measure the decomposition caused by heating monosaccharides with resin, solutions were made up containing 0.04 mg. per ml. of glucose, glucuronic acid, galactose-mannose (1:1), and cellobiose, and 0.2 mg. per ml. of fucose. Five-milliliter aliquots of these solutions were heated with 0.4 gram of resin in sealed glass tubes at 100° C. for 48 and 96 hours. In order to correct for the small amount of colored material eluted from the resin under these conditions, 5 ml. of water and 0.4 gram of resin were also heated under the same conditions. The tubes were tied to a metal plate, which was rotated slowly by means of a motor and pulley system situated outside the oven, so that the contents of the tubes were continuously mixed throughout the period of heating.

The treated solutions were filtered when cool and aliquots were taken from the filtrates for analysis by the carbazole–sulfuric acid method. The solutions obtained after heating with resin were slightly yellow. Absorbances were measured at 548 mμ for the untreated and resin-treated solutions and the solutions obtained from resin and water. The readings for the resin-treated sugar solutions were corrected for the "resin blank" (Table I).

Quantitative Determination of Carbohydrate in Connective Tissue Extracts. The connective tissue extracts (fractions II) had been prepared in this laboratory from Achilles tendon, lung, skin, cartilage, and bone of cattle (*4, 6*). The tissue was extracted with 0.5*N* sodium hydroxide in the cold for 4 days. The extract was neutralized, concentrated, and fractionated with alcohol. Acid mucopolysaccharides (fraction I) were precipitated by addition of alcohol to a concentration of 63%, and fraction II was obtained after the alcohol concentration had been increased to 84%.

The main problem was to determine whether the occurrence of protein in these samples (fractions II) would interfere with the carbazole determination. The problem was examined in three ways. First, their carbazole absorption curves (400 to 600 mμ) were compared with a similar curve for a solution containing 0.04 mg. each of galactose, mannose, and fucose per ml. of solution, to see whether the absorption maxima as well as the shapes of the curves were the same (Figure 2). Secondly, 2 ml. of a standard solution of galactose-mannose (1:1) containing 0.025 mg. of each per ml. was added to 2 ml. of the solutions of connective tissue extracts used above, and then the absorbances were measured at 548 mμ (Table II) to estimate recovery of the added sugar. Thirdly, because the monosaccharide residues in these extracts must be bound to one another or to amino acid residues, the carbazole determinations were also carried out on their hydrolyzates.

The absorbances at 548 mμ for solutions of galactose-mannose in the ratios 2 to 1 and 1 to 2, at total sugar concentrations of 0.03 mg. per ml., fell on the straight line shown in Figure 1. Thus within the range of concentrations studied the total hexose may be determined in a mixture without exact knowledge of the ratios of the different hexoses present.

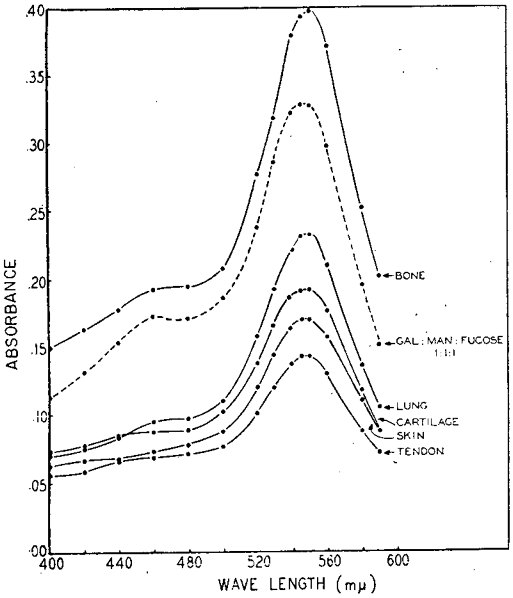

Figure 2. Carbazole–sulfuric acid absorption curves

0.04 mg. per ml. of galactose, mannose, and fucose. 2.41, 1.01, 0.40, 2.06, and 1.61 mg. dry weight per ml. of extracts from bone, lung, cartilage, skin, and tendon, respectively

Table II. Corrected Absorbances (Carbazole) of Solutions of Connective Tissue Extracts, Standard Galactose-Mannose, and a Mixture of Both

	Tissue Extract Concn., Mg. Dry Weight/Ml.	Corrected Absorbances			
		Tissue extract	Gal.-man. soln.	Calcd. for mixture	Exptl. for mixture
Tendon	1.612	0.142	0.180	0.161	0.163
Skin	2.060	0.170	0.180	0.175	0.172
Cartilage	0.403	0.194	0.180	0.187	0.179
Lung	1.014	0.234	0.180	0.207	0.204
Bone	2.414	0.400	0.180	0.290	0.293

Table III. Corrected Absorbances (Carbazole) of Solutions of Connective Tissue Extracts before and after Hydrolysis

	Tissue Extract Concn., Mg. Dry Weight/Ml.	Corrected Absorbances				
		Unhydrolyzed solution	Resin Hydrolyzed, Hours		0.5*N* HCl hydrolyzed, 4 hr.	0.5*N* H_2SO_4 hydrolyzed, 4 hr.
			48	96		
Cartilage	0.4	0.182	0.184	0.177	0.176	0.179
Bone	1.5	0.268	0.271	0.265	0.255	0.239

Carbazole Estimations on Hydrolyzed Connective Tissue Extracts. Five-milliliter volumes of solutions of the extracts from cartilage and bone containing 0.4 and 1.5 mg. per ml., respectively, were heated with 0.4 gram of resin at 100° C. for 48 and 96 hours. Controls were also set up with water and resin alone to correct for the small amount of material leached out of the resin. For comparison, 8 and 30 mg. of the extracts from cartilage and bone, respectively, were heated with 10 ml. of 0.5*N* hydrochloric acid for 4 hours at 100° C.; similar samples were heated under the same conditions with 10 ml. of 0.5*N* sulfuric acid. These conditions using mineral acid are an average of those used by several workers for hydrolyzing similar material. All these hydrolyses were carried out in sealed glass tubes, which were continuously rotated in the oven as previously described.

The tubes were cooled, then the solutions filtered. In the case of resin hydrolyzates, aliquots were taken directly from the filtrates for carbazole analysis. However, in the case of hydrolyzates from hydrochloric acid and sulfuric acid, 5-ml. aliquots of the filtrates were neutralized with 0.6*N* sodium hydroxide, then made up to 10 ml. with water. The results are summarized in Table III.

RESULTS AND DISCUSSION

Effect of Heating Sugars with Hydrogen Form of Cation Exchange Resin. The absorbances (Table I) for solutions of fucose,

Table IV. Absorbances and Hexose Content of Connective Tissue Extracts

	Tissue Extract Concn., Mg. Dry Weight/Ml.	Absorbances			Corrected Absorbance	% Hexose
		Carbazole blank	Unknown blank	Sugar reading		
Tendon	1.612	0.023	zero	0.167	0.144	
		0.028	0.002	0.170	0.140	2.4
Skin	2.060	0.028	0.005	0.203	0.170	
		0.028	0.006	0.203	0.169	2.2
Cartilage	0.403	0.023	0.002	0.218	0.193	
		0.028	0.003	0.225	0.194	13.0
Lung	1.014	0.023	0.012	0.267	0.232	
		0.028	0.013	0.277	0.236	6.3
Bone	2.414	0.028	0.027	0.454	0.399	
		0.024	0.028	0.452	0.400	4.5

glucose, cellobiose, and a mixture of galactose and mannose (1:1) agree well before and after treatment with the resin for 48 or even 96 hours. Thus it is concluded that these monosaccharides are not significantly decomposed by the treatment with resin. On the other hand, glucuronic acid must have been almost completely destroyed. When the solution of glucuronic acid was heated without resin under similar conditions, the same absorbances were obtained as after resin treatment. Thus the effect on glucuronic acid is due to the heating and not to the presence of the resin.

These findings are in agreement with those previously reported on a qualitative basis from results obtained by visual estimation of the intensities of spots on paper chromatograms (*3*), except that fucose is now shown to be stable under the conditions of hydrolysis.

Determination of Carbohydrate in Connective Tissue Extracts. It was known from previous paper chromatographic analysis that these connective tissue extracts all contained galactose, mannose, and fucose (*6*). The absorption curves plotted for these glycoproteins were therefore compared with the curve for a mixture of galactose, mannose, and fucose (Figure 2). These curves have the same shape and the same absorption maxima at 548 mμ. This result is taken as partial evidence that the noncarbohydrate components of these substances do not interfere significantly with the carbazole determination.

In order to measure the recovery of sugar, a known amount of galactose-mannose (1:1) was added to the solutions of connective tissue extracts and carbazole determinations were carried out. The experimental absorbances of the mixtures were compared with the calculated values, which were the arithmetical means of the absorbances found for the extract (Table II, column 3) and the pure galactose-mannose solution (Table II, column 4). The excellent agreement between the calculated and experimental values indicates that the carbazole readings for these substances are not affected by the other constituents present in them or released during the procedure.

While the above determinations were done on untreated connective tissue extracts, further carbazole determinations were also carried out on their hydrolyzates, to determine whether the same results would be obtained when the monosaccharide units were available in the free form instead of being bound in some unknown manner in the original material. Table III shows excellent agreement between the absorbances of the unhydrolyzed solutions and those hydrolyzed with the resin for 48 or even 96 hours. In the case of hydrochloric and sulfuric acid hydrolyzates, there is also good agreement with the unhydrolyzed solution of cartilage extract, but with both acids the values are lower for the bone extract. The loss of sugar may depend on the material being hydrolyzed with mineral acid and may be due to interaction between sugar and amino acids after their liberation. Such interaction would probably be reduced in the case of the resin, as a large part of the amino acids is held by the resin.

The amount of carbohydrate in these samples was calculated from the equation $D = K\ C\ L$. The extinction coefficient, K, was calculated from the values for D (corrected absorbance) and the corresponding values of C (the total sugar concentration in milligrams per milliliter) as shown in Figure 1 for galactose-mannose (1:1) and galactose-glucose-mannose (1:1:1). L (the light path in centimeters) was unity. These standards were chosen because the connective tissue extracts had been shown by paper chromatographic analysis of their hydrolyzates to contain these sugars in different ratios (*6*); the values for these two standards fell on the same straight line when plotted. The mean value of K was 3.66.

Duplicate determinations of absorbances for the "carbazole blank," "unknown blank," and the final readings on the solutions of connective tissue extracts are given in Table IV, together with the calculated "hexose" percentages. These hexose values are in error, because all the substances analyzed contain fucose, which also produces with carbazole a colored substance absorbing maximally at 528 mμ. However, at 548 mμ the absorption due to fucose (corrected absorbance of 0.044 for a solution containing 0.05 mg. per ml.) is small compared with that for a solution of galactose-mannose of the same concentration (corrected absorbance of 0.180). For this reason the absorption maximum for a mixture of galactose-mannose-fucose (1:1:1) is the same as for a mixture of galactose-mannose (1:1), and occurs at 548 mμ. If the fucose contents of these connective tissue extracts were determined, a correction could be made. Nevertheless, the intensities of spots observed on paper chromatograms (*6*) show that the ratio of hexoses to fucose in these materials is large, and thus the hexose values are probably close to the actual values.

The modified carbazole–sulfuric acid procedure developed in this work makes use of the system of blanks developed by Seibert and Atno (*11*), but is simpler and faster, as only two solutions (instead of three) are added to the tubes. The procedure also incorporates the requirements for optical color development and minimal experimental error established subsequently by Holzman, MacAllister, and Niemann (*9*). As the monosaccharide components of the connective tissue extracts had previously been identified (*6*), it was not necessary, as in the case of previous workers (*7, 8, 10*), to use the method of E ratios as a means of qualitative identification; in this way, more suitable standards were set up for the particular substances under study. The correspondence between the shapes of the absorption curves for the extracts and the standards, the excellent recovery of added sugar, and the fact that the same carbazole readings were obtained for galactose, glucose, mannose, fucose, and connective tissue extracts before and after hydrolysis with the resin afford good evidence that the carbazole method is capable of giving an accurate value for the amount of bound hexose present in these particular substances.

ACKNOWLEDGMENT

The work was supported by a grant from Eli Lilly & Co. to C. P. Leblond. Excellent technical assistance by Imre Sarlos is acknowledged.

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RECEIVED for review June 24, 1955. Accepted October 31, 1955.

Study of the Iodide Titration for Determination of Palladium

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A systematic study has been made of a volumetric method for the determination of palladium in palladium-rich alloys. The method is based on the precipitation of palladium as palladous iodide, using the precipitate as its own indicator. This cloud method has been found to have an accuracy comparable with the present gravimetric methods and is especially applicable as a "go-or-no-go" test.

THE need for further systematic study of methods of separation and determination of the platinum metals, which was recognized at the Sixth Annual Summer Symposium in 1953 (*9*), is especially true in the case of palladium. The increasing use of this metal and its alloys in the arts and industry has created a demand for a rapid method for its determination.

The most common determinations of palladium in alloys are the gravimetric methods in which the palladium is separated and weighed as the dimethylglyoximate complex (*8*), or in which the palladium is separated and precipitated as palladous iodide and converted to the metal before weighing (*11*). Recent publications by Atkinson and others (*2, 3*) describe a direct volumetric method based on the titration of palladous chloride with potassium iodide using the precipitate as its own indicator. The usefulness of this method in laboratories with limited equipment and the possibility of its adoption as a control procedure in hallmarking were considered adequate justification for a systematic study of its limitations and accuracy.

REAGENTS

Standard Palladous Chloride (chloropalladous acid). Exactly 1 gram of palladium sponge was dissolved in 4.5 ml. of concentrated hydrochloric acid, 0.9 ml. of concentrated nitric acid, and 10 ml. of water in a covered, lipless beaker. The solution was evaporated almost to dryness, taken up with 10 ml. of $6N$ hydrochloric acid, and evaporated again. After one additional evaporation the residue was taken up with 16.3 ml. of $6N$ hydrochloric acid and diluted to 1 liter.

Ferrous Sulfate Solution. Twenty grams of ferrous sulfate crystals, $FeSO_4.7H_2O$ was dissolved in 100 ml. of distilled water with a few drops of concentrated sulfuric acid. Fresh solution was made every 2 or 3 days.

Standard Potassium Iodide Solution, $0.01N$. Pure potassium iodide (1.660 grams) was dissolved to make 1 liter in distilled water that had been deoxygenated by bubbling an inert gas (argon) through it for 2 hours. The solution was kept in a dark bottle with a tightly fitting stopper and was treated with inert gas at the end of each day. The solution was standardized against pure palladium foil or sponge each day.

EXPERIMENTAL

Analytical Method. A complete description of the analytical procedure is given by Atkinson (*2*). For this study a sufficient quantity of standard chloropalladous acid solution was prepared and aliquot portions were used throughout. In each run, 10 ml. of chloropalladous acid, 5 ml. of concentrated hydrochloric acid, and 15 ml. of 20% ferrous sulfate solution were mixed in a 250-ml. Erlenmeyer flask and titrated with $0.01N$ potassium iodide solution. About 0.5 ml. less than the expected amount of iodide solution (18.74 ml.) was run in slowly while the system was kept well stirred. The flask was stoppered and shaken vigorously for 1 minute, after which about 5 ml. of the solution was transferred to a centrifuge tube and centrifuged at or above 8500 ft. per min. for 15 seconds. One drop of the iodide solution was added to the clear solution in the centrifuge tube and the intensity of the cloud of palladous iodide judged, using a diffuse light background. The contents of the tube were returned to the flask and the titration and testing continued until the addition of 1 test drop failed to give a recognizable cloud in 1 minute.

Sensitivity of End Point. Attempts were made to improve the sharpness of the end point by studying the coagulation of the main precipitate, the illumination of the cloud, and other operating factors. Coagulating agents, such as trisodium phosphate, albumen, and nitrobenzene, and one wetting agent, Duponol WE, were tried; but vigorous shaking followed by centrifuging, as described above, still gave the best clarification of the mother liquor.

Artificial light was found to be less satisfactory than diffuse daylight for illuminating the cloud. A "diffuse light cell" suitable for detecting the cloud under all conditions was made by supporting the centrifuge tube in the center of a beaker which had onion skin paper taped to a portion of its inside surface as a background. The cloud could be seen best when the test drop of potassium iodide was allowed to fall directly on the surface of the liquid from a short distance, rather than run down the side of the tube.

An induction period of about 15 seconds was needed for the precipitate to form well enough to be used as an indicator; development of the cloud for a period of longer than 1 minute did not improve the accuracy of the titrations. It was found that the preferred time for the cloud to form and the choice of the particular degree of diffuse illumination varied with the individual analyst. The effect of such variables was eliminated by having each operator do his own standardization.

Verification of End Point. In spite of the good agreement between the experimental and calculated end points, it was felt that some other confirmation of the end point in addition to visual observation of the formation of the precipitate was desirable. Adsorption effects might be made apparent by comparing the usual end point with that obtained on reversing the order of titration. Although the end point of the reverse titration was not sharp because of insufficient contrast between the color of the added drop and that of the cloud, the titer indicated that there was no great error due to adsorption. Additional evidence for the absence of adsorption effects is found in a comparison of this method with the corresponding method for silver (*6*). In the silver titration the solution is turbid at the end point instead of clear, because of the peptizing action of iodide ions on silver iodide. No similar peptizing action has been observed in the numerous iodide titrations of palladous chloride made in this laboratory. Because peptizing action cannot take place without adsorption, the absence of any peptizing may well indicate that the end point of the palladium titration is not affected by adsorption. Incidentally, the adsorption indicators fluorescein and eosin did not appear to be applicable in this titration.

A potentiometric titration, similar to that of Müller and Stein (*10*), was performed using an atmosphere of argon gas, a gold-calomel electrode system, and a sensitive direct current potentiometer. The titration of the mixture of chloropalladous acid and ferrous sulfate with potassium iodide at room temperature and at 50° C. gave meaningless results. A titration at 50° C. with potassium iodide of chloropalladous acid without ferrous sulfate resulted in a potentiometric end point which agreed with the visual and calculated end points. It is thus apparent that this procedure for palladium, which depends upon the presence of ferrous sulfate, does not lend itself to a potentiometric technique.

Solubility of Palladous Iodide. A search of the literature revealed no data concerning the solubility of palladous iodide in water. A saturated solution of palladous iodide, with a portion of the standard chloropalladous acid diluted to a concentration of 1 γ of palladium per 0.05 ml. of solution, was tested with stannous chloride and with nickel dimethylglyoximate according to Feigl (*5*); both tests were negative for the palladous iodide solution. If the limits of sensitivity of these spot tests for palladous ion as reported by Feigl are correct, palladous iodide has an estimated solubility in water somewhat below 2×10^{-3} gram per liter. The maximum error introduced by this solubility is equivalent to 0.06 ml. of 0.01*N* potassium iodide in a titer of about 20 ml. Even this small error is avoided by direct standardization of reagents against pure palladium metal.

The possibility that the solubility of palladous iodide in excess potassium iodide might affect the titration was examined. Such interference seemed improbable, however, in view of the finding of Beamish and Dale (*4*) that up to ten times the calculated amount of potassium iodide could be added to a precipitate of palladous iodide without danger of palladium loss. The reaction

$$PdI_2 + 2KI \rightleftharpoons K_2PdI_4$$

was studied by adding excess potassium iodide to palladous iodide until a visible red color formed and until a portion of the precipitate appeared to dissolve. Under these conditions more than six times the volume of potassium iodide needed for precipitation was necessary to shift the equilibrium to the right.

Oxidation-Reduction Considerations. In a titration involving iodide it is obviously important to avoid the presence of oxidizing agents such as residual aqua regia from the dissolving of the palladium alloys. On the other hand, the reducing agent used to destroy the residual aqua regia should not reduce or react with the palladous chloride. Hydroxylamine hydrochloride, which is used in some analytical procedures for decomposing residual aqua regia, appeared to cause some complexing or reduction of the palladium, because titers were about 10% low when it was used. Arsenious acid also lowered the iodide titer. Stannous chloride dissolved the precipitate of palladous iodide, so was not considered further. Ferrous sulfate solution did not interfere with the titration and did effectively remove any ordinary excess of aqua regia used in dissolving the alloys.

The ferrous ion, however, would be expected to react with the palladous ion according to the reaction (*7*)

$$2Fe^{++} + Pd^{++} \rightarrow Pd^0 + 2Fe^{+++}$$

This was found to occur at high concentrations of ferrous ion, at temperatures above room temperature, and at low acid concentrations. Therefore the ferrous sulfate should not be added to the palladium solution until after the hydrochloric acid has been added and the resulting mixture cooled to room temperature. The concentration of the ferrous sulfate in the titration mixture should not exceed 100 grams per liter; in some cases, as when aqua regia is absent from the palladium solutions, this concentration should be lowered to 75 grams per liter.

Effect of Other Metals. Tests were performed to determine the effect of interfering metals by introducing solutions of the metal ions into the chloropalladous acid before titration and by actual analysis of palladium-rich alloys. A compilation of work done for this paper and of work reported elsewhere (*2*, *3*) shows that the following metals do not interfere with the accuracy of the titration when present up to 5% by weight of the palladium: bismuth, cobalt, copper, chromium, iridium, iron, manganese, molybdenum, nickel, platinum, rhodium, ruthenium, tin, and tungsten.

Gold ordinarily interferes with this titration by competing with the palladium for the iodide ions. It was found that, if the ferrous sulfate solution was added to the palladium-gold solution and the mixture allowed to stand for 1 hour at room temperature, the gold, accompanied by some palladium, was precipitated when the solution contained 3.6 grams of hydrogen chloride per liter, but that only the gold precipitated if the solution contained about 70 grams of hydrogen chloride per liter. Preventing the interference of gold is thus another good reason for the use of ferrous sulfate in the presence of a high concentration of hydrochloric acid.

Silver also interferes with this determination. Such interference can be partly obviated by filtering off the insoluble silver chloride after dissolving the alloy in aqua regia; some palladium, however, is occluded by the precipitate. It is preferable to determine the palladium by a modified technique without removing the silver (*3*).

"Go-Or-No-Go" Method. The development of the cloud method in the analysis of palladium suggested its investigation as a test such as would be used in the hallmarking analyses of metals for jewelry. Runs were performed in which one drop less than the exact amount of 0.01*N* potassium iodide needed for complete precipitation was added to a 10-ml. portion of the standard chloropalladous acid solution (with the hydrochloric acid and ferrous sulfate). After centrifuging, one more drop of potassium iodide should give a cloud of precipitate, thereby indicating the presence of enough palladium to pass the test. In another series of runs the simulated sample solutions contained not quite enough palladium to pass the test.

Table I. Deviation of Experimental Titers from Calculated Titer of Potassium Iodide Necessary to React with Standard Chloropalladous Acid Solution

	Method		
	Analytical	"Go-Or-No-Go"	Both
Number of runs	41	69	110
Runs within 0.05 ml. of end point (±2.5 parts per thousand)	29 (70.7%)	65 (94.2%)	94 (85.5%)
Runs within 0.10 ml. of end point (±5.0 parts per thousand)	36 (87.8%)	67 (97.1%)	103 (93.6%)

ANALYSIS OF TITRATION DATA

Analytical Data. The statistical analysis of a large number of determinations under optimum conditions for the ordinary volumetric method is given in Table I. The spread of values for the titrations corresponds roughly to that to be expected from probability considerations. For a given volume of the standard chloropalladous acid solution, the average volume of the potassium iodide at the end point, the median value, and the theoretical end point coincide. The distribution of the end point values of the titrations appears to indicate identical tendencies toward high and low results.

"Go-Or-No-Go" Data. Statistical analysis of a number of runs by this method is also given in Table I. The total number of runs, 69, represents 38 runs in which an exact or excess amount of palladium was present and 31 runs in which an insufficient quantity of palladium was present. The distribution of the results from these tests likewise resembles a probability curve, but in this case the skewness of the curve (*1*) is +0.002, indicating a slight tendency to pass samples containing insufficient palladium. These analyses are normally run in triplicate, however, and such a scheme would guard against an occasional erratic titer. Almost 95% of the runs gave results within 2.5 parts per thousand of the correct value, which is satisfactory accuracy for platinum group metal analyses.

DISCUSSION

The various aspects of this somewhat novel application of a cloud method have been investigated. It has been shown that the best operating conditions for achieving a sharp end point involve vigorous shaking after titration, adequate centrifuging, and a suitable light cell for observing the cloud. The method has been shown to have an end point corresponding to the stoichiometric value and free of any great interference from adsorption of solubility effects. The method is of especial application as a "go-or-no-go" test, and with care in operation has an accuracy of ±0.25%, which is comparable to the usual gravimetric methods.

Some precautions to be observed involve maintaining the pH at a low level and the ferrous ion concentration below 15 to 20 grams per liter in the mixture to be titrated with potassium iodide. The advantage of being able to determine palladium without separation from interfering metals may be offset by care that must be taken in observing the cloud end point. It is hoped that this study will stimulate further development of this work toward greater accuracy and will result in increased interest in cloud techniques for other analyses.

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RECEIVED for review September 6, 1955. Accepted January 9, 1956.

Polarographic Determination of Traces of Bismuth, Iron, Lead, Antimony, Nickel, Cobalt, and Manganese in Refined Copper

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A procedure for the polarographic determination of some impurities in refined copper has been developed. The iron and the bismuth are precipitated from an ammoniacal solution. Iron is determined in a tartrate supporting electrolyte, and bismuth is determined in the same solution after the addition of fluoride ions. The other impurities—lead, antimony, cobalt, nickel, and manganese—are separated from the copper by controlled potential electrodeposition and determined in a fluoride supporting electrolyte.

THE conductivity and other properties of copper are adversely affected by the presence of small traces of impurities. The determination of the exact concentration of these trace elements is, therefore, of primary importance. Numerous procedures for analysis have been devised, the majority of which are based on standard chemical methods. Because of the small concentrations of these impurities (refined copper is at least 99.9% pure, while blister copper is approximately 99% pure) it is necessary, when performing a complete analysis, to start with a very large sample. In the case of refined copper as much as 500 grams is used. This must be handled in smaller lots which are recombined after the preliminary separations. Furthermore, most precipitations have to be repeated two or three times to eliminate adsorption errors, and this usually makes standard methods of analysis long and tedious.

Therefore, a simpler and more rapid method of analysis has been developed, which includes all the most important impurities in copper. The determination of selenium and tellurium having been previously investigated in this laboratory (*2*), the present research was confined to the determination of bismuth, iron, lead, antimony, manganese, cobalt, and nickel.

APPARATUS AND REAGENTS

A Leybold-Nachfolger polarograph was used. This apparatus was slightly modified to ensure better moving electrical contacts at the ends of the potentiometric wire on the axle of the potentiometric drum and at the sliding contact. In the first case the surface of contact was enlarged, while in the second, the small wheel whereby the contact is made was replaced by a fixed loop of platinum wire.

The electrolysis cells were of the simplest type with mercury pool anodes. They were immersed in a thermostat adjusted to 25° ± 0.05° C. by means of a Sunvic thermostat (Type T.S. 1) and vacuum switch relay (Type F 102/4).

All chemicals used were reagent grade.

The complete analysis for the seven impurities listed above was carried out in two parts. Iron and bismuth were determined in one sample, and lead, antimony, manganese, cobalt, and nickel were determined in another sample.

DETERMINATION OF IRON AND BISMUTH

A sample of 30 grams of refined metal or 10 grams of blister copper is weighed out and dissolved in a minimum volume of 1 to 2 nitric acid. Any excess acid is evaporated and the solution diluted to 100 ml. with distilled water. Sufficient ammonium hydroxide is added to precipitate the bismuth and ferric hydroxides, and to form the copper-ammonia complex. Five grams of ammonium carbonate is added and the solution left on a water bath for 12 hours. It is then filtered, and the precipitate is immediately washed as free as possible of copper with a very dilute solution of ammonium hydroxide and ammonium carbonate (*7*).

The filter paper and the precipitate are transferred to a 50-ml. borosilicate glass beaker, which is then placed in a muffle furnace. The paper is burned off at as low a temperature as possible and the residue ignited at 400° C. for 10 minutes to destroy the organic material. This residue contains all the iron and the bismuth plus traces of the other minor elements.

Exactly 10 ml. of a supporting electrolyte, $0.3M$ with respect to sodium potassium tartrate and containing 0.002% methyl red (pH 7.6), is added. The solution is very gently warmed to aid in dissolving the residue, then electrolyzed after the removal of oxygen by bubbling with nitrogen. The resulting polarogram gives a well-defined wave for iron between -0.3 and -0.4 volt *vs.* the saturated calomel electrode.

The applied potential is now set to a value just high enough to obtain the limiting current for iron, and very small quantities of solid sodium fluoride are added, with shaking, until the current due to the reduction of the iron has been completely suppressed. The solution is electrolyzed after the removal of oxygen, and a curve is obtained showing a wave for bismuth alone at -0.5 volt *vs.* the saturated calomel electrode.

The concentrations of the iron and bismuth are determined from standard curves. The solutions used in the preparation of these curves were made up under the same conditions as the unknown samples.

DETERMINATION OF LEAD, ANTIMONY, NICKEL, COBALT, AND MANGANESE

A second sample of 30 grams of refined copper or 10 grams of blister copper is weighed out and dissolved in the minimum volume of 1 to 2 nitric acid. Two milliliters of sulfuric acid is added and the solution evaporated until fumes of sulfur trioxide are evolved. The residue is dissolved in 50 ml. of water and another 2 ml. of concentrated sulfuric acid is added. The lead sulfate precipitate is filtered off, washed carefully, and set aside for later use. The filtrate and washings are united in a 150-ml. beaker and the solution is diluted or evaporated to 100 ml. This solution is then electrolyzed between platinum electrodes at a cathode potential of −0.51 volt against an electrode of saturated mercurous sulfate in 1*N* sulfuric acid. The electrolysis is continued until the current has fallen to a steady value. The platinum cathode is then removed and carefully washed, and the washings are collected in the electrolysis vessel. A clean cathode is placed in the solution and the electrolysis continued for 30 minutes.

The solution is evaporated to 20 ml., transferred to a smaller beaker, and evaporated to dryness. The filter paper containing the lead sulfate precipitate is added to the residue in the beaker, which is placed in a muffle furnace. The paper is burned off at as low a temperature as possible and the residue ignited to 400° C. for 10 minutes.

After cooling, exactly 10 ml. of a supporting electrolyte, 0.1*M* with respect to sodium fluoride and containing 0.05% gelatin, is added. The beaker is gently warmed to effect solution of its contents, cooled to 25° C., and the solution electrolyzed after the removal of oxygen. The resulting curve shows well-defined waves for any residual copper and for nickel, cobalt, and manganese. These waves are sufficiently well spaced to prevent any overlapping at the concentrations of the metals found in refined or blister copper.

Nitric acid is now added to the solution, a known volume just sufficient to dissolve the lead fluoride without reducing the pH much below 3, which would cause the lead and antimony waves to overlap. The oxygen is removed and the solution electrolyzed. The polarogram obtained shows waves for lead and antimony which are suitable for the quantitative determination of these two elements. The exact concentrations are again determined from standard curves obtained by preparing standard solutions of the elements as described above.

The total time required for carrying out a complete analysis of the seven elements is about 14 hours, which includes 12 hours spent in the preparation of the bismuth and iron solution. This represents a considerable saving in time compared with days required for a similar analysis using standard methods. The quantities of substances used are also very much smaller.

The precision, which is of the order of 4 to 5%, seems generally better than that obtained by different analysts using the same standard methods. It is satisfactory considering that the maximum concentration of any of these elements is 0.02%.

DISCUSSION OF RESULTS

Separation of Copper and Bismuth. In developing this procedure, attempts were made to separate the copper from the bismuth by controlled potential electrodeposition from acid tartrate solutions and from nitric and sulfuric acid solutions (*4*, *5*). In the first case it was found that the amounts of tartrate which would have to be added to keep the large quantities of copper in solution would be prohibitively large. In the second case it was found that, although it was comparatively easy to reduce the concentration of the copper to 10^{-2} gram-atom per liter, it would take a very long time to reduce the concentration of the copper any further without also reducing the bismuth. Also, this would probably require two separate electrolytic separations.

Attempts were then made to determine the bismuth in a solution containing 10^{-2} gram-atom of copper per liter by making use of the differential or the derivative polarographic methods.

In spite of the synchronization of the dropping rates by the method of Airey and Smales (*1*), the differential method proved to be unsuitable. It was not possible to obtain reproducible curves at the galvanometer sensitivity required for the determination of the small quantities of bismuth present, when a total compensation of the copper wave was attempted. The derivative method, using the simple circuit of Leveque and Roth (*3*), which is essentially the same as that of Vogel and Riha (*8*), seemed more promising at first; greater separation could be achieved by this method than by classical polarography. Two distinct peaks were obtained for bismuth and copper up to concentrations of $10^{-3}M$ in copper; however, at greater concentrations of copper, its peak completely masked the bismuth peak. The method is not suitable, therefore, for this determination where the concentration of copper is so much greater than that of bismuth. However, in the case of bismuth and antimony in 1*N* hydrochloric acid, the derivative curves showed two distinct peaks which were proportional to the respective concentrations of these two metals. It is impossible to measure the wave height accurately when the conventional waves are used.

Because of these difficulties, bismuth was separated from copper by the chemical method described above. This enables the electrodeposition of copper in the resulting solution to be carried out more rapidly, because it is possible to work at a higher potential and bismuth is deposited simultaneously with the copper.

Use of Sodium Fluoride as Supporting Electrolyte. This electrolyte was found more suitable and pleasant to use than pyridine hydrochloride. Excellent waves were obtained for manganese, but cobalt and nickel at first gave ill-defined waves in spite of the use of fresh gelatin as recommended by West and Dean (*9*). It was subsequently found that the shape of these waves was affected by the concentration of the sodium fluoride solution and the time of standing after mixing the reducible substance and the supporting electrolyte. The best curves were obtained only after prolonged standing (48 hours), but after a period of 45 minutes they are reasonably satisfactory and suitable for quantitative determinations. The optimum concentration of the electrolyte is approximately 0.15*M*. The curves are most deformed in concentrated solutions, which also show the greatest time effect.

Cobalt and nickel give poor waves at a pH below 7, while the antimony wave is displaced to more negative potentials with an increase in the pH and finally disappears at a pH greater than 6. Lead fluoride is sparingly soluble in neutral fluoride solutions but gives an excellent wave at lower pH's. Below pH 3 the antimony and lead waves tend to overlap; therefore, the sodium fluoride solutions should not be made too acid if these two metals are to be determined simultaneously.

In some regions zinc is found as an impurity in the copper; this was not the case in the samples investigated here. However, when present, zinc would interfere in the determination of nickel because the half-wave potentials for nickel and zinc in fluoride solutions are −1.12 volts and −1.14 volts, respectively. Work is being completed in this laboratory and will be published shortly on the determination of zinc in the presence of nickel, cobalt, manganese, and iron in fluoride solutions. In addition, Meites (*6*) has developed a method for the analysis of nickel and zinc in copper.

ACKNOWLEDGMENT

A. J. Eve is indebted to the South African Council for Scientific and Industrial Research for the granting of a research bursary, without which this work could not have been carried out. Both of the authors are also indebted to Roan Antelope Copper Mines, Ltd., for furnishing suitably analyzed samples of refined and blister copper.

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Received for review May 3, 1955. Accepted December 8, 1955.

Detection and Paper Chromatography of Sugars and Sugar Phosphates in Picric Acid System

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Paper chromatography with the *tert*-butyl alcohol–picric acid–water solvent system has been limited to sugar phosphates. Its application to sugars in general has now been made possible by the development of a simple spray technique for their detection on paper chromatograms. The reaction involves the reduction of alkaline picrate to picramic acid or other reddish-brown products. Various sugars and sugar phosphates (including glyceraldehyde, erythrose, and glyceraldehyde 2- and 3-phosphate) have been examined in this system, and their R_f and R_{ribose} values determined.

THE *tert*-butyl alcohol–picric acid–water solvent system has been used for the separation and identification of various sugar phosphates by paper chromatography (*3*). In this procedure the chromatograms are developed, after spraying the paper with a perchloric acid–molybdate reagent, by appropriate treatment leading to the formation of reduced phosphomolybdate in the areas containing phosphate (*2*). Reducing sugars are not readily detected in the presence of picric acid by the standard spray techniques such as ammoniacal silver nitrate (*5*) or aniline oxalate or phthalate (*6*).

It has now been found that the areas containing sugar or sugar phosphate are easily made visible after the dried chromatogram is sprayed with ethanolic sodium hydroxide and heated. Under these circumstances the picric acid remaining in the paper reacts with the sugar or its alkaline decomposition products and forms a reddish-brown spot, probably picramic acid (*4*, *7*), against the yellow background of the sodium picrate.

Several sugars including glyceraldehyde, erythrose, arabinose, ribose, xylose, rhamnose, fructose, galactose, glucose, mannose, lactose, maltose, sucrose, and raffinose, as well as some of their phosphoric acid esters, were examined by descending paper chromatography in the picric acid system using the new development technique. The R_f values of most of the substances studied are sufficiently different to allow their separation and identification by the procedures described. The R_f and R_{ribose} values found are presented in this paper.

EXPERIMENTAL

Apparatus. A borosilicate glass cylinder, 24 cm. in diameter and 46 cm. in height, covered with a tight-fitting glass plate was used. The paper and the solvent trough for descending chromatography were supported on a stainless steel rack. A container with solvent was placed in the bottom of the jar to provide a saturated atmosphere of the vapor phase. The data presented were obtained with Whatman No. 1 filter paper, but in several instances where Schleicher and Schuell No. 597 filter paper was used, similar results were found.

Solvent. A solution was used consisting of 4 grams of picric acid dissolved in 80 ml. of *tert*-butyl alcohol and 20 ml. of water (*3*).

Materials. With the exception of glyceraldehyde and erythrose, the sugars were the usual commercial preparations. Ribose 5-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, and glucose 6-phosphate were purchased as barium salts from Nutritional Biochemicals Corp., Cleveland, Ohio. Cyclohexylammonium 2-phosphoryl D-glyceraldehyde dimethyl acetal (hexylammonium-2-*o*-phosphonyl-D-glyceraldehyde dimethyl acetal) was converted to glyceraldehyde 2-phosphate by hydrolysis at 40° C. for 96 hours after treatment of a 2% solution with excess Dowex 50, acid form (*1*). Glyceraldehyde was obtained in solution from calcium glyceraldehyde 3-phosphate by hydrolysis at pH 4 with purified semen phosphatase.

The erythrose was in the form of an aqueous solution.

Procedure. Samples of 10 µl. containing 0.02 to 0.3 µmole of sugar or sugar phosphate were applied to the starting line of the paper, which was exposed to the vapor phase of the solvent for 3 to 5 hours. The solvent was then added to the trough through a hole in the glass plate, which could be closed by means of a rubber stopper. After 16 to 26 hours at room temperature, the paper sheets were removed from the cylinder, allowed to dry in air for about 30 minutes, sprayed with a freshly prepared solution consisting of equal parts of $1N$ sodium hydroxide and 95% ethanol, and heated in an oven at 85° C. for 7 to 10 minutes. The areas containing sugar appeared as reddish-brown spots, which retained their characteristic appearance for several weeks.

Table I. Paper Chromatography of Sugars and Sugar Phosphates[a] in Picric Acid System

	Range	
Sugars	R_f	R_R[b]
Glyceraldehyde	0.25-0.32	0.60-0.64
Erythrose	0.49-0.60	1.2-1.4
L-Arabinose	0.38-0.39	0.74-0.78
Ribose	0.37-0.49	1.0
Xylose	0.43-0.45	0.84-0.90
L-Rhamnose	0.46-0.48	1.1-1.2
Fructose	0.31-0.34	0.72-0.76
Galactose	0.24-0.28	0.56-0.57
Glucose	0.33-0.35	0.65-0.67
Mannose	0.32-0.37	0.74-0.79
Lactose	0.09-0.11	0.21
Maltose	0.12-0.15	0.29-0.30
Sucrose	0.22	0.51-0.52
Raffinose	0.06-0.07	0.13-0.15
Sugar Phosphates		
Glyceraldehyde 2-phosphate[c]	0.33-0.40	0.77-0.79
Glyceraldehyde 3-phosphate	0.19-0.26	0.44-0.52
Ribose 5-phosphate	0.46-0.49	0.90-0.92
Fructose 6-phosphate[d]	0.34-0.39	0.82-0.90
Fructose 1,6-diphosphate[d]	0.32-0.34	0.76-0.82
Glucose 6-phosphate[d]	0.25-0.27	0.60-0.62

[a] All sugars and sugar phosphates, with the exceptions noted, were of the D- configuration.

[b] $R_R = \frac{\text{distance traveled by compound}}{\text{distance traveled by D-ribose on same chromatogram}}$

Values shown are the lowest and highest found on at least two chromatograms.

[c] The dimethyl acetal from which glyceraldehyde 2-phosphate was obtained gave R_f and R_R values of 0.79 to 0.83, and 1.9, respectively.

[d] The free acid prepared by treatment of the salt with Dowex 50, acid form, gave identical R_f values.

RESULTS AND DISCUSSION

The *tert*-butyl alcohol–picric acid solvent moves with two fronts, a faster colorless one and a slightly slower picric acid front, which remains visible on drying. The picric acid front was used for the calculation of the R_f values. The ranges of these values for the respective compounds are shown in Table I. In each instance at least two chromatograms were prepared and run on different days.

The relatively wide ranges of R_f values shown for some compounds—e.g., ribose and erythrose—were found over a period of 2 to 3 months, during which there was considerable fluctuation between day and night temperatures. This probably accounts for the wide variation found in these cases. Ribose was present on many of the chromatograms showing variations in R_f values; therefore, it was possible to calculate R_{ribose} values, or the ratio

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of the distance traveled by the particular compound to that traveled by ribose. These R_R values were much more uniform, and chromatograms were prepared for all the substances studied, in comparison with ribose. The resulting R_R values are also given in Table I. In some instances compounds which appeared to have similar R_f values—e.g., erythrose and rhamnose—could be distinguished when compared with ribose.

A single, characteristic spot was found for each of the compounds studied, with the exception of the nonreducing sugars sucrose and raffinose, and the hexose phosphates. The first two compounds showed evidence of slight hydrolysis during a run, and the hexose phosphates gave faint "shadows" as described by Hanes and Isherwood (*3*). In these instances, and with the other sugar phosphates studied, the respective spots were also located on separate chromatograms by means of the perchloric acid–molybdate spray (*2*). No significant differences in R_f or R_R values were found.

APPLICATION TO OTHER CHROMATOGRAPHIC SYSTEMS

Reducing sugars can also be detected in systems where picric acid is not used, such as acetone–boric acid (*8*), by spraying the chromatogram with a fresh solution containing picric acid (1 gram), 1*N* sodium hydroxide (25 ml.), and ethanol (25 ml.), then heating as described above.

ACKNOWLEDGMENT

The authors would like to express their thanks to C. E. Ballou, University of California, Berkeley, for samples of glyceraldehyde phosphates, and to A. S. Perlin, National Research Laboratories, Saskatoon, Sask., Can., for the sample of erythrose. The semen phosphatase was prepared in this laboratory by Forrest H. Riordan, III. This investigation was supported by research grants from the American Cancer Society, the Rockefeller Foundation, and the United States Public Health Service.

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RECEIVED for review November 18, 1955. Accepted December 12, 1955.

Peroxytrifluoroacetic Acid as a Reagent for Determination of the Carbonyl Function in Aldehydes and Ketones

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The Baeyer-Villiger reaction of simple aliphatic aldehydes and ketones is quantitative with peroxytrifluoroacetic acid in ethylene chloride solution and affords a new method for the determination of these compounds. Samples of aldehydes or ketones are treated with excess standard peroxytrifluoroacetic acid, the reacton is allowed to proceed to completion, and the residual peroxy acid is determined iodometrically. The method is accurate to ±10% in the most unreliable cases investigated and recoveries high by 3% are most often obtained. A procedure for the preparation of standard anhydrous solutions of peroxytrifluoroacetic acid in ethylene chloride is described.

BECAUSE the most commonly employed method for the quantitative estimation of the carbonyl function in aldehydes and ketones is based on the slow and reversible oximation reaction (*3*), a new procedure was sought, which might be executed volumetrically with high precision. Such a method has been made available through the discovery of the extremely reactive oxidizing agent, peroxytrifluoroacetic acid, and the preparative oxidation of ketones to mixtures of esters (Baeyer-Villiger reaction) by this reagent has been recently described (*2*).

The basis of this method lies in ease with which solutions of peroxytrifluoroacetic acid containing trifluoroacetic acid enter into the Baeyer-Villiger reaction (*1*) with aldehydes and ketones. As these reactions

$$\mathrm{RCOR' + CF_3CO_3H \rightarrow RCOOR \text{ and } R'COOR + CF_3CO_2H}$$

are relatively rapid and peroxytrifluoroacetic acid solutions in ethylene chloride lose their active oxygen only very slowly, these determinations may be carried out by the simple iodometric titration of excess peroxy acid after a reasonable reaction time (up to 1 hour at 50° C.) without the use of a reagent decomposition blank determination.

Solutions of peroxytrifluoroacetic acid may be conveniently prepared by the reactions of a weighed sample of 90% hydrogen peroxide with the calculated volume of a standard solution of trifluoroacetic anhydride in ethylene chloride. Under these conditions the reactions

$$\mathrm{H_2O_2 + (CF_3CO)_2O \rightarrow CF_3CO_3H + CF_3CO_2H}$$

$$\mathrm{H_2O + (CF_3CO)_2O \rightarrow 2CF_3CO_2H}$$

rapidly take place and the resulting standard solution of peroxytrifluoroacetic acid will retain its active oxygen titer throughout a normal working day.

SOLUTIONS AND REAGENTS

Ethylene Chloride. Three liters of U.S.P. ethylene chloride was washed three times with concentrated sulfuric acid and three times with water, and dried over sodium sulfate. This material was then distilled through a helices-packed 2-foot column until water ceased to be removed from the system by azeotropic distillation. Sufficient phosphorus pentoxide was added to the boiler to remove any extraneous moisture and the distillation continued in a system protected from atmospheric moisture. Two liters of material boiling at 84° C. was collected in a flask and protected from atmospheric moisture.

Standard Trifluoroacetic Anhydride Solution. Two hundred grams of trifluoroacetic anhydride (obtained from the Minnesota Mining and Manufacturing Co.) was slowly distilled into the ethylene chloride described above, using a distillation system

Table I. Condition and Results Obtained for Ketone and Aldehyde Determinations

Carbonyl Compound	Time, Min.	Temp., ° C.	Mmoles Used	Mmoles Found	% Carbonyl Compound Found
Methyl ethyl ketone	30	25	5.65	5.85	103
			2.26	2.33	103
			1.87	1.93	103
			1.46	1.51	103
Cyclopentanone	30	25	2.41	2.51	104
			2.71	2.74	101
Cyclohexanone	30	25	2.08	2.11	102
Cycloheptanone	30	25	1.83	1.88	103
Methyl *n*-hexyl ketone	30	25	1.58	1.64	104
Methyl cyclopropyl ketone	30	25	1.91	1.77	93
	120	25	1.91	2.03	106
Methyl *n*-butyl ketone	30	25	1.60	1.68	105
			1.60	1.70	106
Methyl *n*-heptyl ketone[a]	30	25	1.41	1.64	116
	20	25	1.41	1.62	115
Acetone	60	50	2.10	2.08	99.2
n-Dodecyl aldehyde	20	50	1.31	1.46	111
n-Butyraldehyde	60	50	1.62	1.55	96.0
Isobutyraldehyde	60	50	2.24	2.26	101
	20	50	2.24	2.12	94.5

[a] This high reproducible value is probably due to impure ketone.

which was protected from atmospheric moisture. The resulting solution was then drawn into a dry, evacuated 25-ml. Karl Fischer buret which had a 3-liter reservoir and was rigorously protected from atmospheric moisture by a drying tube on the air inlet. Solutions prepared in this manner remain standard indefinitely. Ten-milliliter aliquots of this anhydride solution were run into ice water and titrated to the bromothymol blue end point with standard 0.2*N* sodium hydroxide solution.

Hydrogen peroxide was a 90% solution supplied by the Buffalo Electrochemical Co. Approximately 1-gram samples were weighed into 100-ml. volumetric flasks and made up to volume with distilled water and aliquots were titrated with 0.1*N* permanganate solution in the presence of 25% sulfuric acid. Tests showed that the particular lot used throughout this work consistently contained 87.6% hydrogen peroxide.

PREPARATION OF STANDARD PEROXYTRIFLUOROACETIC ACID SOLUTION

About 1 gram of standardized hydrogen peroxide was accurately weighed into a 100-ml. volumetric flask. Moles of water and hydrogen peroxide present in the sample were calculated and the volume of standard trifluoroacetic anhydride solution equivalent to the sum of the water and hydrogen peroxide present was added from the buret. The two-phase mixture rapidly reacted, with the evolution of heat to produce a solution containing the peroxy- acid and trifluoroacetic acid. Because of the volatility of the peroxy- acid it was found advisable to dispense it as needed from a 10-ml. microburet carrying a 5 ml. reservoir and a drying tube. Several aliquots were analyzed by delivering 10 ml. of this solution to 25 ml. of ice-cold 25% sulfuric acid containing 1.0 gram of potassium iodide. Iodine was liberated immediately and was titrated with 0.1*N* sodium thiosulfate which had been standardized with potassium dichromate by the usual procedure. Peroxy- acid solutions prepared in this manner showed no change in titer during a normal 8-hour working day or on heating at 50° C. for 1 hour.

Aldehydes and ketones were Eastman Kodak white label or of equivalent purity. Samples were distilled immediately prior to use.

PROCEDURE

An approximate 2-gram sample of the ketone or aldehyde is weighed into a 25-ml. volumetric flask and made up to the mark with ethylene chloride. A 2-ml. aliquot is transferred to a small iodine flask and cooled by an ice-water bath. Ten milliliters of the peroxy- acid solution is run in the iodine flask and the resulting solution allowed to stand at 25° to 50° C. for 30 minutes. During the mixing of the ketone and peroxy- acid solutions a slight rise in the temperature usually occurs, which may bring the precooled solution to near the desired final temperature. Particularly sluggish aldehydes or ketones (such as acetone) require warming to 50° C. for 60 minutes in a tightly stoppered iodine flask.

At the conclusion of the reaction period, the iodine flask is cooled with an ice-water bath, 25 ml. of ice-cold 25% sulfuric acid is added, followed by 1.0 gram of potassium iodide, and the liberated iodine is rapidly titrated with standard 0.1*N* sodium thiosulfate solution until the ethylene chloride layer is colorless. The difference between the titer of the 10 ml. of standard peroxy-acid and the titer of the reaction mixture is used to calculate the equivalents of carbonyl group present.

$$\frac{N \text{ thiosulfate (titer of 10 ml. of stand. soln.} - \text{titer of reaction mixture)}}{2} = \text{milliequivalent carbonyl}$$

DISCUSSION

Unfortunately, the method appears to be limited to aldehydes and ketones of the aliphatic type. In preliminary experiments using acetophenone and phenyl cyclohexyl ketone the peroxy-acid evidently attacked the aromatic ring of the ester product (phenylacetate or phenylcyclohexanecarboxylate) to produce highly colored solutions with the consumption of more than the theoretical amount of peroxy- acid. For this reason the emphasis of this work was placed on aliphatic compounds. Table I records the results obtained for 12 aliphatic aldehydes and ketones.

The results shown in Table I indicate both the accuracy and reproducibility of the method. The high values found in many cases may be attributed to the loss of peroxytrifluoroacetic acid by volatilization during the analytical procedure or in some cases to the impurity of the carbonyl compound. It is felt, however, that this method presents a considerable improvement over those commonly employed and that specific analytical procedures may be developed for unusual cases.

The ease of preparation of peroxytrifluoroacetic acid recommends its use in the determination of olefins and nitrosamines, and these applications are under examination.

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RECEIVED for review November 9, 1955. Accepted January 14, 1956.

Solvent Removal by Vacuum Evaporation

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Volatile solvents can be removed without loss of solute by vacuum distillation, in which bumping is prevented by imparting a swirling action to the solution. Up to 10 test tubes can be used simultaneously, or almost any vacuum-resistant container up to 1000 ml. in size. Flasks can be filled over half full. Continuous solution feeding is possible with one of the devices described.

WITH the devices described here it is possible to concentrate solutions to dryness at reduced temperatures, in the absence of air and without loss, using a wide variety of containers. The basic apparatus, described previously (*5*), uses a swirling motion to prevent splashing during the vacuum distillation. The following modifications increase its versatility, ease of use, and compactness.

Figure 1 illustrates the arrangement used for high vacuum distillation. *A* is a size 13 rubber stopper attached to the shaft of a variable speed motor fitted with a gear reducer (such as the A. H. Thomas Co. stirrer, No. 9242). The 8-mm.-diameter hole in the stopper accommodates the solid glass rod (6 × 80 mm.) of the adapter, *B*. *B* has a 24/40 male joint on the bottom and a side arm 8 mm. in outside diameter and 15 mm. long. *D* is a connector, with a 24/40 female joint on top, and one to 10 legs, each fitted with a neoprene stopper. It is useful to have an assortment of connectors with two, six, and 10 legs, with different sizes of rubber stoppers. When fewer than two, six, or 10 containers are used, empty tubes are attached to make up the difference. The six- and 10-leg connectors are made readily from 50-ml. Erlenmeyer flasks having a 24/40 joint. Part *D* can be replaced by a flask fitted with a 24/40 joint. Greasing the connection between *B* and *D* improves the vacuum.

C is a length of rubber tubing, about $^{3}/_{16}$ inch in inside diameter and $^{3}/_{8}$ inch in outside diameter, which is twisted 4 times and attached at each end to the arms of a metal ⊔. The ⊔ (not shown) is 3.5 inches between arms and 9 inches deep; it is attached by a clamp to a heavy ring stand, which also supports the motor. Part *D* or a flask is inserted between the twisted tubing, which supports the weight while permitting a swirling action to take place. It is important to choose a grade of rubber tubing which is strong but reasonably elastic; otherwise it is difficult to insert the glassware.

E is a pot of water on a thermostatically controlled hot plate. The hot plate sits upon a support of adjustable height (such as the Cenco-Lerner Lab-Jack). Circular pots minimize splashing. *F* is a 9-inch length of rubber pressure tubing, $^{1}/_{4}$ inch in inside diameter, connecting *B* and a rubber stopper which is inserted into the wide mouth of the condenser flask, *G*. *G* is a combination condenser and safety trap which is cooled by an isopropyl alcohol–dry ice mixture contained in a Dewar flask, 145 mm. in inside diameter and 500 mm. deep. The short inner tube of the condenser portion permits the condenser under favorable conditions to fill up to its mouth, a total volume of 1150 ml. The attached trap has a long inner tube to ensure complete condensation of vapors which escape the condenser part. Two wooden covers are used over the mouth of the Dewar flask to minimize condensation of atmospheric moisture, because ice makes the slurry of isopropyl alcohol–dry ice become sludgy. The slurry should cover the shoulder of the condenser. (The condenser can be used in lyophilization, in which case the slurry level should be below the mouth of the large entrance tube, and tube *F* should be replaced by a short piece of large-bore tubing.)

H, connected to *G* by a short rubber tube, lets air into the system when the evaporation is finished. A capillary constriction slows down the air to prevent carryover of the contents of *G* into the pump. The bubbler on *H* is convenient for signaling when the vacuum is completely broken. *J* is a 250-ml. bulb inserted into the line to prevent liquid condensate from splashing up into the pump from a prematurely filled trap. When splashing is noticed, the pump is stopped and the plug in the top of *J* is removed to let air in without forcing solvent into the pump.

K is a bypass with a capillary tube and a 4-mm.-bore vacuum stopcock, intended to prevent splashing of the evaporating solution when the pump is started. The stopcock is kept closed until the dissolved air has been pumped from the solution, or, in the case of low-boiling solvents, until the solution temperature has gone down sufficiently. This takes from $^{1}/_{4}$ to 5 minutes, depending on the volume, solution, and speed of swirling. If splashing occurs even with the stopcock closed, the capillary should be constricted further by softening at the middle with a hand torch. With certain solutions, the entire distillation must be run with the stopcock closed to avoid foaming.

L is a tube filled with potassium hydroxide pellets, which are supported by an extra coarse sintered-glass disk, 50 mm. in diameter; this protects the pump against acidic vapors. The bottom of *L* is connected to a vacuum pump (such as the Welch Duo-Seal pump, Model 1400-B) with pressure tubing $^{7}/_{16}$ inch in inside diameter.

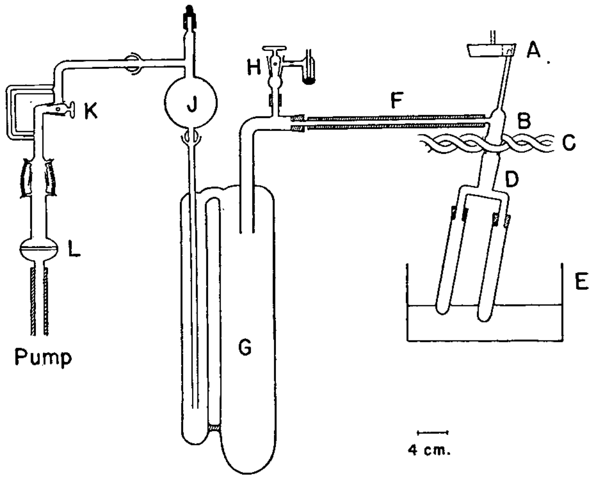

Figure 1. Apparatus for evaporation under high vacuum

A.	Size 13 rubber stopper
B.	Adapter, 24/40 male joint
C.	Rubber tubing
D.	Connector, 24/40 female joint, with one to 10 legs
E.	Heated water bath
F.	9-inch length of rubber pressure tubing, $^{1}/_{4}$-inch i.d.
G.	Condenser–safety trap combination
H.	Air inlet and bubbler
J.	250-ml. bulb
K.	Bypass
L.	Potassium hydroxide trap

Experience has shown that a surprisingly high swirling speed must be used if splashing is to be uniformly avoided. For some arrangements there is an optimum speed that gives maximum motion; this speed should be adjusted occasionally as the volume of solution decreases. The water bath should not be brought up to the tubes until the solution temperature has gone down appreciably, as evidenced usually by condensation of atmospheric moisture on the tubes. However, in the case of solvents which freeze readily, such as water, the bath must be raised before the solution freezes. The bath temperature is usually kept below 40° C.; too high a temperature causes splashing or incomplete condensation and premature filling of the safety trap. The entire apparatus may be placed on a cart.

When *G* becomes full, it is allowed to warm (but never by pouring hot water on the cold glass) and most of the contents are poured out. A certain amount cannot be removed this way and it is necessary to aspirate out the rest with a rubber tube inserted through the large opening. The safety trap inner tube is then rinsed with a little acetone, which is removed; air is then sucked through until the inner tube is dry. If the condenser is very full, thawing will cause some of the condensate to flow out of the mouth; in this case, the thawing should take place in the sink. If the water bath is not raised or the heat not turned on, the large inner tube of *G* will freeze shut when water is being distilled. This is remedied by disconnecting *F*, squirting in some glycerol (rather than a less dense solvent), and raising *G* a bit. Glycerol is also used to lubricate the point of contact between *A* and *B*.

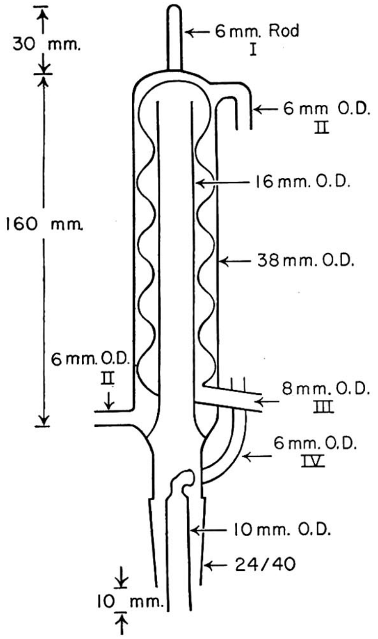

Figure 2. Swirler condenser for evaporation under aspirator vacuum

When a water aspirator can be used as the vacuum source, it is possible to use parts *A* and *F* as described by connecting *F* to a long, efficient water-cooled condenser. The bottom of the condenser is connected by a short, downward sloping rubber tube to the aspirator. By using the condenser shown in Figure 2, it is possible to conserve a great deal of work space and speed the distillation.

The condenser fits directly in the flask (or *D*, Figure 1) and moves with the flask just like *B*. The solid rod, I, fits inside the stopper, *A*. Tubes II are for the cooling water. Tube III carries the condensate via an 8-inch length of pressure tubing ($^{1}/_{4}$ inch in inside diameter) to a short vertical glass condenser whose lower end connects by a short, downward sloping rubber tube to the aspirator. The short condenser need be only about 6 inches long; it serves to condense vapors missed by the swirling condenser. Tube IV is closed at the top by a small rubber bulb or, when large volumes must be concentrated, is connected to a solution reservoir via a 6-inch length of neoprene tubing, $^{3}/_{16}$ inch in inside diameter with a $^{1}/_{16}$-inch wall. The tubing should be treated with chloroform–ethyl alcohol in a Soxhlet extractor if organic solvents are to be added. A screw clamp or stopcock on the reservoir controls the flow rate to match the evaporation. For the reservoir, a separatory funnel (the Fischer & Porter Ultramax funnel with Teflon stopcock is good for organic solvents) or an aspirator bottle having 5-mm. tubing sealed onto the outlet may be used. The lower part of tube IV is widened to help deaerate the incoming solution and prevent splashing up into the condensing section. It is well to grease the joint between the condenser and flask (or *D*) if a good vacuum is desired.

With this arrangement, it is usually unnecessary to evacuate slowly at the beginning, but the flask should be swirling before the aspirator is turned on. With 500- and 1000-ml. flasks, smoother swirling action is obtained by inserting the flask near one end of the tubing twist, *C*, and placing an extension clamp under the tubing close to the other side of the neck. The clamp is attached to the lower part of the metal ⊔ by a clamp holder and is slipped back when not needed; in effect, it shortens the length of *C*.

As with the high vacuum arrangement, the water bath (contained in a 10-inch-diameter pot) is seated on a thermostatic hot plate and is raised into position by a Lab-Jack. In setting up the apparatus, the distillate should be kept flowing down to prevent impairment of the vacuum.

In a test run, using a 1000-ml. flask half filled with water and placed in a 63° C. water bath, a distillation rate of 340 ml. per hour was observed. The water inside remained at room temperature. Higher distillation rates have been obtained by using a condenser larger than the one shown in Figure 2 (22-mm. inside tube, 45-mm. outside tube) and also by eliminating tube IV. When continuous evaporation is carried out, higher distillation speed results when the liquid level in the flask is kept low.

A basic consideration in designing the swirler-condenser is the need to have a short, wide-bore tube to carry the vapors; subsequent travel paths, which carry the much smaller volume of condensate, can then be relatively narrow.

Other devices for vacuum evaporation without capillary tubes have been described (*1–4, 6*), and several models are available commercially. Some of the devices described in this paper might be useful in conjunction with the other designs. Features of this design are (1) lack of rotary seals; (2) use of ordinary stirring motor which can be used for other purposes; (3) the fact that the high vacuum arrangement can be used for other vacuum work, such as ordinary distillation or lyophilization, without changing the trap; (4) the wide variety of vessels which can be used (standard test tubes, Erlenmeyer flasks up to 125 ml., flat-bottomed boiling flasks up to 300 ml., round-bottomed boiling flasks up to 1000 ml.); (5) the fact that more than one of the smaller vessels can be used simultaneously; and (6) the ability to fill vessels, other than test tubes, more than half full.

ACKNOWLEDGMENT

The author wishes to express his appreciation to John A. D. Cooper for suggestions on design. The glassware was made by the H. S. Martin Co., Evanston, Ill., and the Delmar Scientific Laboratories, Chicago, Ill.

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RECEIVED for review October 13, 1955. Accepted February 1, 1956.

Electrophoresis Cells Modified for Determination of Labeled Protein Fractions

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A method for observing the amount of certain radioactive isotopes, such as phosphorus-32, calcium-45, sulfur-35, and carbon-14, incorporated in a particular protein fraction has been developed. The ascending arm of the center cell of a Tiselius electrophoretic apparatus is modified so that the radioactivity associated with the labeled protein can be measured as the protein fronts migrate past the observation point. The usual information gained from such an experiment—electrophoretic pattern, protein mobilities, and concentration of the separated protein fractions—is not affected by this modification.

A COMBINATION of electrophoretic and radiochemical techniques has been used to determine the amount of phosphorus-32 in the various electrophoretic components of blood serum (*2, 4*) and egg yolk (*3*). Recently, to determine smaller amounts of phosphorus-32 activity and to detect radiations from the radioactive isotopes calcium-45, sulfur-35, and carbon-14, the center section of the Tiselius electrophoresis cell has been modified further.

MODIFICATION OF CELLS

The construction of the first cell modification (cell *A*), in which a semicylinder was ground from one of the arms of the center cell, has been discussed (*4*). In the original apparatus, the thinnest point contained 0.9 mm. of glass between the counter tube and the active samples. These cells have been ground further, so that as little as 0.2 mm. of glass remains at this point. This has resulted in increased counting rates, and no serious structural weakness has developed in the cells.

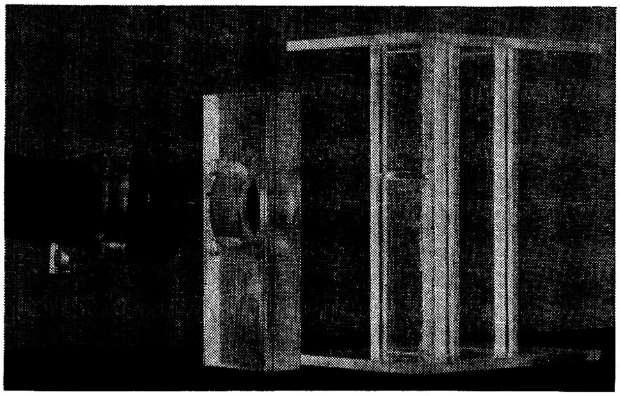

Figure 1. Center electrophoresis cell, Lucite block, and end-window tube dismantled

The present center-cell modification (cell *B*), illustrated in Figure 1, was constructed as follows:

A slit was ground through the cell wall with Carborundum coated, rubber-based disks (0.75 mm. thick and 2.5 cm. in diameter) mounted on a dentist's drill. The slits, for the two cells prepared in this manner, were 1.5 mm. wide and 15 mm. long. In determining the position of the slot with reference to cell height, two factors must be considered: resolution of electrophoretic components and convenience of assembly of equipment. For the cells described the compromise positions were approximately $^2/_5$ the distance from one end of the cell. This gave sufficient component resolution, while allowing easy assembly of the counter tube with the cell and its associated equipment. One side of a polystyrene film, with an areal density of 2.7 mg. per sq. cm., was coated with a thin coat of Lubriseal and placed on the inner surface of the channel of the electrophoresis cell. The excess grease was removed from the portion of the polystyrene film exposed by the slit. The polystyrene film extended the full length of the cell and no turbulence in the protein fronts was noted in subsequent electrophoreses.

A Lucite block with a fitting to hold an end-window counter tube was centered over the slit and glued to the glass with Glyptal cement No. 1276. As an additional precaution, thin strips of Scotch electrical tape were employed to hold the ends of the Lucite block to the glass cell. An end-window counter tube

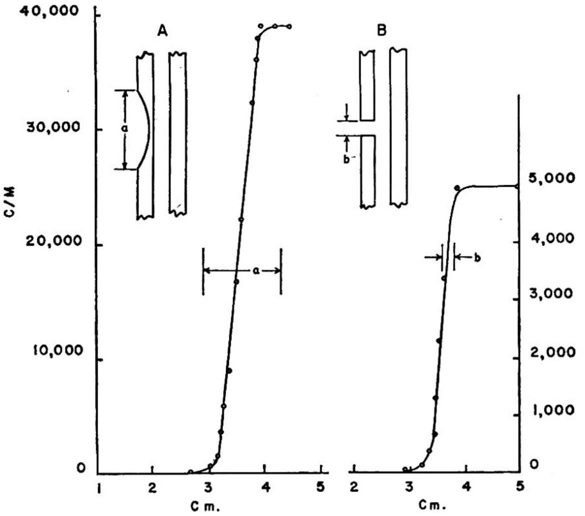

Figure 2. Difference in total counting rates and resolution with a standard phosphorus-32 solution for two cell assemblies

A and *B* measure 90% of total increase in counting rate in linear distances of 7 and 4 mm., respectively

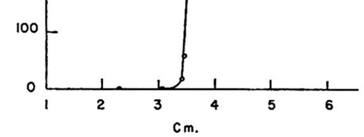

Figure 3. Resolution of cell assembly *B* for calcium-45 solution

90% of total increase in counting rate is detected in linear distance of 2 mm.

(Nuclear Instrument and Chemical Corp. Model D-35) was fastened to the Lucite block, so that the tube window fitted against the glass cell. An O ring seal prevented water from entering the space between the polystyrene film and tube window. This assembly is illustrated in Figure 1. The high voltage lead to the counter tube was encased in flexible rubber tubing; the joints were covered with Scotch electrical tape and finally sprayed with Krylon plastic spray.

RESULTS AND DISCUSSION

Results obtained by using the first modification have been described (*2–4*), and data collected with the second modification will be available soon (*1*). This communication compares the usefulness of both modifications for phosphorus-32 and demonstrates the sensitivity of cell *B* for calcium-45.

The data shown in Figure 2 were obtained by adding a solution containing phosphorus-32 to the unmodified column of the cells and measuring the activity as the solution rose in the adjacent column and passed the counter tube. To reduce the effect of scattering, a small amount of hexane was floated on the surface of the solution. *A*, Figure 2, is an illustration of how the counting rate changed as the phosphorus-32 passed the semicylinder (chord length *a*) ground in the side of cell *A*, whereas *B* of Figure 2 shows the rise in activity as the same solution passed the slit in cell *B* (slit width *b*).

It is apparent that the counter tube assembly (Raytheon CK 1020) with cell *A* measured 8 times as much phosphorus-32 activity as the end-window tube assembly with cell *B*. This was done, however, at the expense of resolution, for in the first case, 90% of the total increase in counting rate occurred in a 7-mm. rise in level, while in the second case the same percentage was obtained in only a 4-mm. band. This means that although higher activities may be needed, better resolution may be obtained by using cell *B*. Its greater resolving power and lowered counting rate were due to the fact that in the second case the radiations were collimated and the tube was at a greater distance from the radiation source. In addition, the gradually curved surface of the semicylinder of cell *A* allowed some radiations to pass through before the thinnest portion of the cell wall was reached. As was shown in a few cases, a greater resolution could be attained in the case of cell *A* when a thin lead shield containing a slit was placed between the counter tube and the glass cell wall.

In the case of calcium-45, however, a different set of conditions existed. The combined thickness of the counter tube wall and glass wall of cell *A* was such as to preclude its use with low energy beta emitters such as calcium-45, sulfur-35, and carbon-14. A graph of counting rate *vs.* liquid height for a solution of calcium-45 with cell *B* is given in Figure 3. In this case over 90% of the increase in counting rate occurred in a 2-mm. band. With the low energy beta-particle emitters, therefore, it was possible to resolve and measure very narrow bands of activity when cell *B* was used. Counting efficiency factors of 0.007 and 0.002% for calcium-45 and sulfur-35, respectively, have been determined for cell assembly *B*.

Although the amount of glass between the sample and the counter prevented the detection of low energy beta emitters with cell *A*, it proved satisfactory for phosphorus-32, particularly when the electrophoretic peaks were well resolved. The cell *B* modification was better suited for the low energy beta emitters or less well-resolved peaks. Intermediate steps in the development of cell *B* included the fabrication of a Lucite cell similar to cell *B*. Its inferior optical properties, however, caused it to be replaced with cell *B*.

ACKNOWLEDGMENT

The work was supported in part by research grants from Eli Lilly and Co., Indianapolis, Ind., and the United States Atomic Energy Commission.

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RECEIVED for review June 20, 1955. Accepted January 11, 1956. 16th Midwest Regional Meeting, ACS, Omaha, Neb., 1955. Contribution No. 516, Department of Chemistry, No. 57, Department of Physics, Kansas Agricultural Experiment Station.

Total Sulfur in Hydrocarbons by Monochromatic X-Ray Absorption

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A Bureau of Mines study involving the separation and identification of sulfur compounds in petroleum created the need for a rapid and nondestructive method of determining total sulfur on small samples. X-ray absorption methods met these requirements very well. By modifying existing equipment a method has been developed that makes calibration simple and rapid. Monochromatic radiation is obtained by using the fluorescence attachment to an x-ray spectrograph. Samples can be analyzed in about 20 minutes with precision comparable with other x-ray methods as well as some chemical methods.

X-RAY science is about 60 years old. Roentgen performed the first crude analysis by x-ray absorption methods in 1895. The more refined use of x-radiation in qualitative and quantitative chemical analysis has been known since 1923, when von Hevesy (*2*) published a book on the subject. In more recent years, however, further developments in this field have been stimulated by the availability of better commercial equipment and by demands from industry for speedier analytical methods. Total sulfur determination by x-ray absorption has advantages over gravimetric and titrimetric methods because it is more rapid, the precision is good, and the sample is not destroyed. This last factor can be very important to a research chemist who may spend much effort and time isolating small sulfur concentrates from crude petroleum.

DERIVATION OF METHOD

This procedure is based on the well-known law of absorption of monochromatic electromagnetic energy passing through a homogeneous absorber. From the absorption law it is known that

$$\ln I/I_0 = -\mu \frac{w}{a} \qquad (1)$$

where I_0 is the intensity of the beam entering the absorber, I the intensity after passing through the absorber, μ the mass absorption coefficient at a given wave length, a the cross-sectional area of the absorber, and w the weight of the absorbing material.

When the sample is a mixture of materials, μ is calculated from μ_i of each component i and its mass fraction, F_i, as follows:

$$\mu = \Sigma_i \mu_i F_i \tag{2}$$

When the absorber is a pure hydrocarbon, Equation 2 becomes:

$$\mu_{CH} = \mu_C F_C + \mu_H F_H \tag{3}$$

Carbon and hydrogen are reported to have equal mass-absorption coefficients (*5*) at a wave length of 0.53 A. For the conditions used in this laboratory the wave length at which equal absorption occurred was found to be closer to 0.59 A. Hughes (*3*) in 1950 found fairly definite evidence that liquid hydrocarbons in long cells caused scattering of the x-ray beam that resulted in mass absorption coefficients differing as much as 15% from published values.

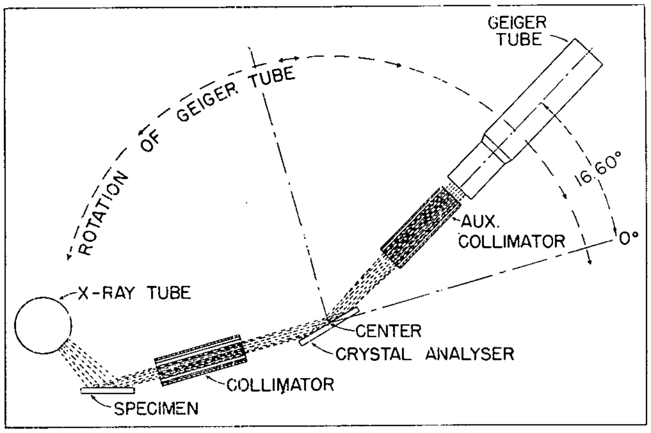

Figure 1. Basic geometry of fluorescent analysis unit

As the absorption of a pure hydrocarbon is independent of the carbon-hydrogen ratio at a wave length of 0.587 A. Equation 3 becomes:

$$\mu_{CH} = \mu_C = \mu_H \tag{4}$$

If sulfur is present in the hydrocarbon Equation 2 combined with 4 gives:

$$\mu_{CHS} = \mu_{CH} F_{CH} + \mu_S F_S \tag{5}$$

where F_S, the mass fraction of sulfur in the hydrocarbon, is the quantity to be measured.

Combining Equations 1, 2, and 5 and 1, 2, and 4 gives:

$$\ln I_{CHS}/I_0 = -(\mu_{CH} F_{CH} + \mu_S F_S)\frac{w}{a} \tag{6}$$

and

$$\ln I_{CH}/I_0 = -\mu_{CH}\frac{w}{a} \tag{7}$$

If $\frac{w}{a}$ is held constant for both hydrocarbon and hydrocarbon plus sulfur, Equation 7 can be combined with Equation 6:

$$\ln I_{CHS}/I_{CH} = -[\mu_{CH}(F_{CH} - 1) + \mu_S F_S]\frac{w}{a} \tag{8}$$

and, as $F_{CH} + F_S = 1$, Equation 8 becomes:

$$\ln I_{CHS}/I_{CH} = -(\mu_S - \mu_{CH})\frac{w}{a}F_S \tag{9}$$

The sulfur-calibration curve is based on Equation 9. At a wave length of 0.587 A., μ_S and μ_{CH} are constants. The cross-sectional area of hydrocarbon and hydrocarbon plus sulfur are constants by virtue of cell construction. The mass of the absorbing material w can be made a constant by weighing identical amounts of samples into the cells. Under these conditions Equation 9 is linear, with its slope essentially $(\mu_S - \mu_{CH})\frac{w}{a}$.

APPARATUS

The apparatus used is a North American Philips x-ray spectrograph unit consisting of a fluorescent analysis unit, a power supply capable of 60 kv. at 50 ma., and a scaler unit.

Figure 1 is a diagram indicating the principle of the fluorescent analysis unit. The primary beam from the tungsten x-ray tube is directed upon a stationary specimen having a fixed angular relationship with respect to both the primary beam and the goniometer axis on which a crystal is mounted. By substituting a sample cell for the first collimator in Figure 1 and moving the crystal and Geiger tube to the proper angle, the absorbed radiation of the desired wave length can be obtained and measured. Figure 2 shows the modifications made to the goniometer to accomplish this. In addition to adding a cell holder which allowed two cells to be placed in the beam alternately, the whole fluorescent attachment was fastened to a tilting table, so that the cells could be held vertically. By taking constant weights of sample and hydrocarbon this cell position eliminated the necessity for density measurements and errors due to temperature variations. A constant mass of material is in the beam regardless of temperature fluctuations during analysis, using a vertical cell partly filled with sample. This vertical cell arrangement is similar to that used with the double-beam x-ray photometer (*4*).

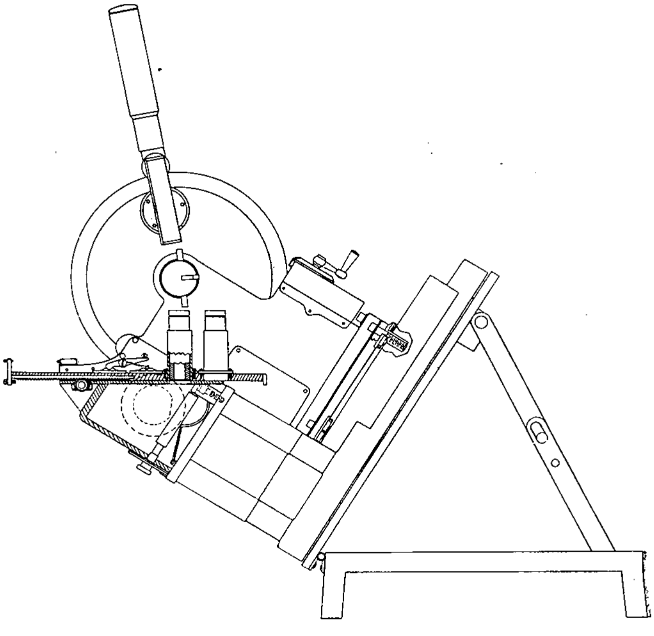

Figure 2. Modifications to fluorescent analysis unit for x-ray absorption analysis

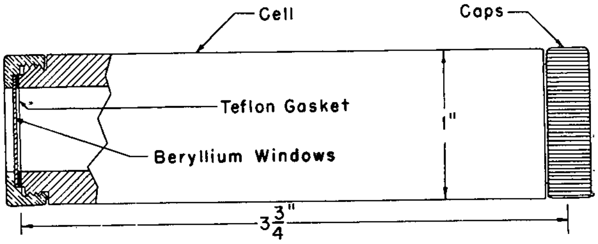

Figure 3. Adsorption cell for x-ray sulfur determination

The cells used in this work are shown in Figure 3. They were constructed from aluminum tubing closed on both ends with brass screw caps fitted with high-transmittance beryllium windows. *Teflon* gaskets prevented leakage or evaporation of the sample. The cells were calibrated by weighing identical amounts of a hydrocarbon into each and comparing the transmittance of x-rays through them.

OPERATING CONDITIONS

A counting rate of 350 counts per second through a cell filled with 8 grams of a hydrocarbon was obtained by adjusting the accelerating voltage at 45 kv. and the current at 35 ma.

Table I. Summary of Experimental Conditions for the Determination of Sulfur

X-ray tube target material	Tungsten
Material in specimen holder	Palladium
X-ray tube accelerating voltage	45 kv.
X-ray tube current	35 ma.
Crystal monochromator	LiF
Goniometer angle	$2\theta = 16.60°$
Scaler operation	Fixed count
Scale factor	256 (25,600 counts)
Wt. of sample	8 grams
Aluminum absorption cell	
Cell length	$3^3/_4$ inches
Inside diameter	$^9/_{16}$ inch
Volume (approximate)	14 ml.
Beryllium window	0.750 inch O.D.
Beryllium window	0.015 inch thick

Table II. Effect of 1% Impurity upon Sulfur Analysis by X-Ray Absorption

Impurity	Calculated as Wt. % Sulfur
Nitrogen	0.02
Oxygen	0.07
Sodium	0.3
Chlorine	1
Calcium	2
Iron	4
Lead	19

As indicated previously, it is necessary to find the wave length at which the mass-absorption coefficients for carbon and hydrogen are identical. Victoreen (*5*) data indicated that this occurs at 0.53 A. Cadmium metal placed in the specimen holder would give a $K\alpha$ radiation near this wave length (0.536 A.). To check the identity of mass-absorption coefficients at this wave length 8 grams of two hydrocarbons with widely varying carbon-hydrogen ratios were weighed into two cells, respectively. Using the scaler the transmittances of each cell was measured. Theoretically, transmittances should have been identical after corrections for cell difference were applied. Identical transmittances were not obtained, however, so the $K\alpha$ radiation of silver of 0.561 A. was used. Transmittances obtained at this wave length were more nearly identical, and by plotting and extrapolating these data it was determined that a wave length of about 0.59 A. was that at which mass-absorption coefficients for carbon and hydrogen were apparently equal. As palladium has a $K\alpha$ radiation of 0.587 A., this metal was used, and the calculations of equal absorbance at this wave length were confirmed experimentally.

A lithium fluoride analyzer crystal was used to diffract the $K\alpha$ radiation of palladium into the Geiger tube. This condition was obtained at a stationary angle of 16.60° (2θ).

Table I summarizes experimental operating conditions for determining total sulfur in hydrocarbon-base stocks.

PROCEDURE

A calibration curve was obtained by comparing 8 grams of cetane in the x-ray beam with 8 grams each of six standard sulfur solutions. The time was recorded for the passage of 25,600 counts through each cell four times, alternating the cetane and the standard cell to minimize intensity fluctuations. A calibration curve was plotted.

Unknown samples were analyzed in the same manner. Eight grams of sample were weighed into a cell and compared in the x-ray beam with 8 grams of cetane or other pure hydrocarbon. The natural log of the ratio of transmittances was applied to the calibration curve to obtain the weight per cent sulfur. A complete analysis requires about 20 minutes.

INTERFERENCES

Any element, other than carbon, hydrogen, and sulfur, present in the sample being analyzed will cause an error in sulfur determination. Most impurities will give high results. Nitrogen and oxygen, the most probable impurities in petroleum fractions, interfere less than most other impurities. Table II lists a few of the elements that might be found as impurities in distillate fuels. The interferences shown were calculated from the mass-absorption coefficients of each element. The interferences of nitrogen and oxygen were checked experimentally, however, and were found to be in very close agreement with the calculated results. As noted in the table, the interference increases with the atomic number of the element, lead giving a large error in the sulfur determination; however, these interferences are not so serious as they might appear. Table III lists some of the impurities for a few distillate fuels that were analyzed by this laboratory. These results represent the extremes in impurity concentrations found in a series of 40 fuels analyzed. The greatest interference found in all fuels analyzed was caused by oxygen. The highest oxygen content, 5100 p.p.m. of oxygen, would be interpreted as 0.03 weight % sulfur.

PRECISION AND RESULTS

An estimate of the maximum error that would be expected from this method considering the statistical counting error at a 95% confidence level as the only variable factor, would be 0.04 weight % sulfur. This value is based on obtaining 102,400 counts and assuming that the terms I_{CHS} and I_{CH} (Equation 9) have equal maximum error. That is,

$$\frac{\Delta \dfrac{I_{\text{CHS}}}{I_{\text{CH}}}}{\dfrac{I_{\text{CHS}}}{I_{\text{CH}}}} = \frac{\Delta I_{\text{CHS}}}{I_{\text{CHS}}} + \frac{\Delta I_{\text{CH}}}{I_{\text{CH}}}$$

The assumption is also made that the coincidence counting errors are compensated by calibration of the equipment over the range of counting rates to be expected.

Table III. Some Distillate-Fuel Impurities

Nitrogen, P.P.M.	Oxygen, P.P.M.	Iron, P.P.M.	Calcium, P.P.M.	Sodium, P.P.M.	Accumulative Interference as Wt. % Sulfur
3650	4470	1.32	0.04	0.18	0.039
430	1930	22.1	0.03	0.35	0.023
430	1480	0.08	0.95	0.25	0.012
80	..	0.29	0.03	0.48	...
400	1400	0.16	0.03	0.33	0.011
400	..	0.93	0.06	0.26	0.001
300	1500	...	0.02	0.16	0.011
200	5100	3.97	0.29	..	0.038
100	2500	0.44	0.03	..	0.018

The precision of the x-ray and bomb results was calculated from the data presented in Table IV. The estimated standard deviation for the x-ray results was 0.036 and for the bomb results 0.074. The *F* test (*1*) applied to these data indicates this difference to be significant at a 95% confidence level.

The sulfur-concentration values listed in the column "known" in Table V were obtained by blending sulfur compounds of established purity with pure hydrocarbons. Using these known values and the results as determined by the x-ray and bomb methods, straight lines were fitted to these data. The known weight per cent of sulfur was the independent variable, and the x-ray and bomb results were the dependent variables. An estimate of the standard deviation of differences between the dependent variables and independent variables was calculated (*1*, *6*). These values are 0.038 for the x-ray results and 0.089 for the bomb results.

SUMMARY AND CONCLUSION

The data presented in this paper show that this x-ray method for total sulfur is comparable with other x-ray methods and certain chemical methods in precision. It has the advantage of rapidity and conservation of samples over chemical methods. It has an advantage over other x-ray methods in the ease and repeatability of obtaining monochromatic radiation. The equipment used, although perhaps too expensive to warrant its purchase for this purpose alone, is easily and economically adaptable to this problem for laboratories that may have the same or comparable equipment available for other work.

Table IV. Data Used to Calculate Precision

Sample	X-Ray, Wt. % Sulfur	ASTM D 129-52 Bomb, Wt. % Sulfur
A	1.928	1.856
	1.955	1.819
	1.910	
	1.928	
	1.867	
	1.918	
B	0.867	0.892
	0.807	0.873
C	3.359	3.099
	3.342	3.275
D	1.556	1.496
	1.465	1.660
E		2.399
		2.330
F		0.594
		0.494
G		0.418
		0.465
Estimated standard deviation	0.036	0.074

Calculated $F = 4.23$
Critical $F_{7,\,8,\,0.05} = 3.50$

Table V. Comparison of Sulfur Analysis by X-Ray Absorption and Bomb Methods

Known Wt. % Sulfur	ASTM D 129-52 Bomb, Wt. % Sulfur	X-Ray, Wt. % Sulfur	Deviation of Bomb from Known	Deviation of X-Ray from Known	Deviation of X-Ray from Bomb
0.272		0.269		−0.003	
0.297		0.352		+0.055	
0.300		0.277		−0.023	
0.345	0.442	0.366	+0.097	+0.021	−0.076
0.387	0.542	0.360	+0.155	−0.027	−0.182
0.513	0.544	0.486	+0.031	−0.027	−0.058
0.526	0.539	0.566	+0.013	+0.040	+0.027
0.765	0.762	0.734	−0.003	−0.031	−0.028
0.816	0.770	0.873	−0.046	+0.057	+0.103
0.824	0.883	0.867	+0.059	+0.043	−0.016
0.892	0.925	0.834	+0.033	−0.058	−0.091
1.014	1.04	1.064	+0.026	+0.050	+0.024
1.052	0.965	1.027	−0.087	−0.025	+0.062
1.054	0.990	1.113	−0.064	+0.059	+0.123
1.202	1.13	1.169	−0.072	−0.033	+0.039
1.513	1.578	1.465	+0.065	−0.048	−0.113
1.688	1.54	1.656	−0.148	−0.032	+0.116
1.809	1.778	1.770	−0.031	−0.039	−0.008
1.907	1.838	1.955	−0.069	+0.048	+0.117
1.989	2.03	1.967	+0.041	−0.022	−0.063
2.295	2.365	2.279	+0.070	−0.016	−0.086
3.114	3.02	3.098	−0.094	−0.016	+0.078
3.176	2.98	3.146	−0.196	−0.030	+0.166
3.333	3.187	3.342	−0.146	+0.009	+0.155
Estimated standard deviation of differences			0.089	0.038	0.097
Calculated t					0.66
$t_{20,\,0.05}$, critical					2.09

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Received for review September 6, 1955. Accepted January 4, 1956.

Oxidation of Platinum Electrodes in Potentiometric Redox Titrations

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Drifting potentials observed in potentiometric redox titrations using platinum electrodes can be explained by oxidation of the surface of the electrode. The formation of the oxide coating and its dissolution are slow processes, dependent on the concentration and nature of the oxidizing and reducing agents present in the solution. Automatically recorded titration curves show an end-point error consistent with the drifting of potentials studied by means of potential-time curves.

NUMEROUS investigators have observed drifting potentials with platinum electrodes in oxidizing solutions (*1*, *2*, *8*). The magnitude of the drifting depends on the nature of the solution and the past history of the electrodes. Forbes and Bartlett (*1*) found that a platinum electrode immersed in a dichromate solution showed a slow drift to more positive (oxidizing) potentials over a period of 43 minutes. Winter and Moyer (*8*), titrating iron(II) with dichromate, found a positive drift in the vicinity of the end point and beyond, which they attributed to the low rate at which the chromium(III)–dichromate system reaches equilibrium. Furman (*2*) mentions a positive drift near the end point in titrating iron(II) with cerium(IV) and permanganate, and a negative drift during the back-titration. He also attributes this drift to the low rate of the chemical reaction in the solution.

Because drifting potentials are observed in titrations which give sharp and permanent end points when indicators are used, it would seem that the drift is caused by a slow reaction of the electrode with the solution and not by a slow attainment of equilibrium in the solution. That platinum electrodes cannot always be considered inert is pointed out by Lingane (*7*). He notes the possibility of attack of platinum electrodes in concentrated chloride solutions by cerium(IV) and permanganate with resulting uncertain potential measurements. Kolthoff and Tanaka (*4*)

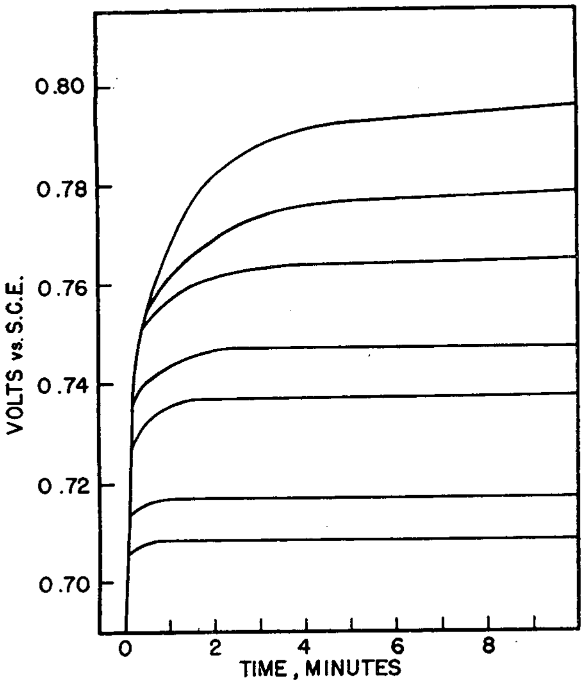

Figure 1. Potential drift of prereduced platinum electrodes in poised mercurous–mercuric perchlorate solutions

1*M* perchloric acid

studied anomalous currents in polarograms taken with rotating platinum electrodes at oxidizing potentials and showed that their results could be explained by the oxidative attack of the electrode with the formation of a layer of platinous oxide. Drifting potentials observed in potentiometric measurements can be explained in a similar manner.

EXPERIMENTAL

Apparatus. A Brown Electronik recorder (10 mv. full scale) was used to record the potentials as a function of time. A Beckman Model H2 pH meter provided a high impedance input to the recorder. A voltage divider and a biasing circuit were provided so that the recorder zero and the potential range could be varied. The recorder was calibrated after each adjustment of zero and potential range by substituting a Rubicon portable precision potentiometer for the cell.

Titration curves were recorded with the same apparatus. Reagent delivery was made using a motor-driven syringe similar to that described by Lingane (*6*). The syringe motor was started and stopped in synchronization with the recorder chart motor, allowing volume measurements to be made in terms of recording time. Syringe delivery rate was 0.94 ml. per minute in this work.

Electrodes were made from C.P. grade platinum wire, 0.81 mm. in diameter (American Platinum Works, 99.98% assay). Wire was sealed in 6-mm. soft glass tubing and cut to a 5-mm. length.

In order to exclude chloride from the solution, a Beckman double-junction calomel electrode was used. The double-junction compartment was filled with 0.6*M* sodium nitrate.

Reagents. All chemicals used were reagent grade. Mercuric perchlorate solutions were prepared by dissolving reagent grade mercuric oxide in excess perchloric acid. Mercurous perchlorate solutions were made from the mercuric perchlorate solutions by shaking the mercuric perchlorate with excess mercury and allowing the solution to stand for 3 days.

The acid concentration of the mercuric perchlorate solutions was checked by adding excess potassium iodide and titrating with standard sodium hydroxide.

RESULTS AND DISCUSSION

Potential *vs.* Time Curves. In order to establish the potential at which drifting begins, potential *vs.* time curves were recorded in poised mercurous-mercuric perchlorate solutions in 1*M* perchloric acid. Perchlorate solutions were used to prevent possible attack of the electrode due to complex ion formation. Any initial oxide layer on the electrode was removed by prereducing the electrode in 0.1*M* ferrous perchlorate for 10 minutes prior to recording. To remove oxygen, nitrogen was bubbled through the solutions for 10 minutes before recording and continuously during the recording period. The recorder was started simultaneously with the immersion of the electrode in the poised solution. The curves obtained are shown in Figure 1. All potentials were measured with respect to the saturated calomel electrode.

In solutions poised at potentials less than about 0.73 volt, the electrode assumed its final potential within 20 seconds. However, when placed in solutions poised at 0.74 volt or higher, drifting potentials were observed. In the most oxidizing solution shown (1*M* mercuric perchlorate) drifting was still detectable after 15 minutes.

Drifting arbitrarily was considered to take place if the potential was changing detectably 1 minute after the electrode had been placed in the solution. Using this criterion, the potential at which drifting begins—i.e., minimum drifting potential—can be placed between 0.72 and 0.74 volt. This minimum drifting potential is close to the platinum–platinous hydroxide potential for 1*M* acid solutions (*5*), and agrees with the cathodic dissolution patterns observed by Kolthoff and Tanaka (*4*). In the region of drifting, the observed electrode potential is a mixed potential, intermediate between the platinum couple potential and the mercurous-mercuric couple potential. As soon as the electrode is oxidized completely, it becomes passive and indicates the equilibrium potential of the solution. The drifting time necessary to reach the equilibrium potential represents the time necessary to coat the electrode completely.

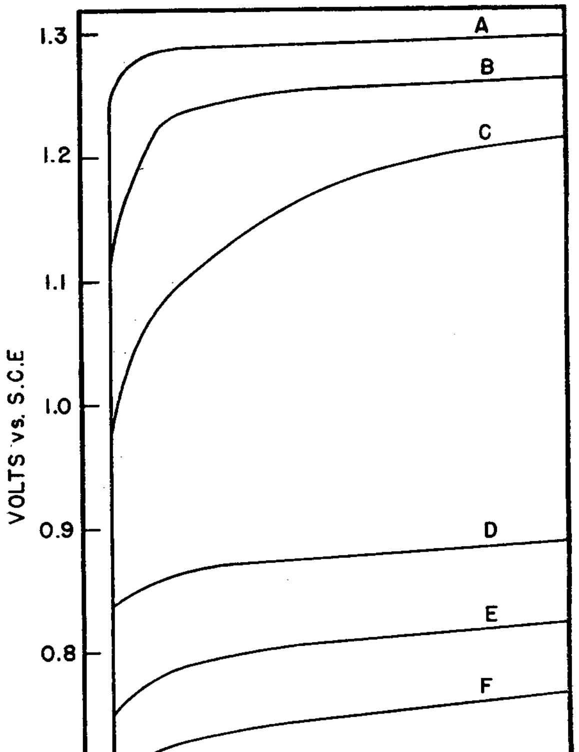

Figure 2. Potential drift of prereduced platinum electrodes in oxidizing solutions

1*N* sulfuric acid

A. 0.1*M* Ce(IV)
B. 0.01*M* Ce(IV)
C. 0.001*M* Ce(IV)
D. 0.1*M* dichromate
E. 0.01*M* dichromate
F. 0.001*M* dichromate

Curves with mercurous-mercuric solutions in 0.1*M* perchloric acid showed no shift in minimum drifting potential when compared with curves obtained in 1.0*M* acid. It was not possible to investigate the shift at pH values above 1 because of hydrolysis of the mercuric ion.

The effect of dichromate and cerate solutions on prereduced electrodes also was determined by recording curves in the manner described above (Figure 2). Oxidation of the platinum is apparent from the drifting potentials. The more strongly oxidizing solutions require less time to form the oxide layer. More concentrated solutions of a given oxidizing agent form the layer more quickly. These data, plus the fact that the platinum electrode is the only reducing agent in the system, indicate that the drifting observed in potentiometric redox titrations is due to the oxidation of the electrode rather than a slow titration reaction.

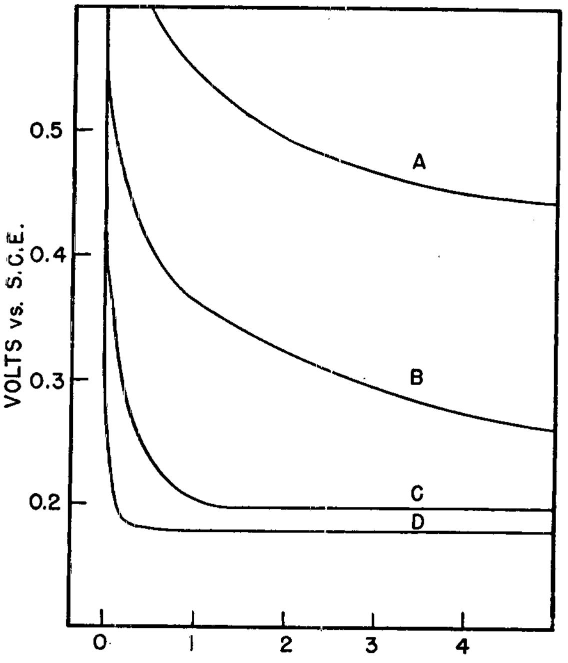

Figure 3. Potential drift of preoxidized platinum electrodes in reducing solutions

1*N* sulfuric acid

A. $10^{-5}M$ Fe(II) *C.* $10^{-3}M$ Fe(II)
B. $10^{-4}M$ Fe(II) *D.* $10^{-2}M$ Fe(II)

The behavior of electrodes which were preoxidized before being placed in a reducing solution also was studied (Figure 3). The electrodes were oxidized by placing them in 0.1*M* cerate solutions for 10 minutes. They were then placed in dilute iron(II) solutions (1*N* sulfuric acid) and potential *vs.* time curves were recorded as before. Equilibrium potentials were achieved much more rapidly under these conditions, even though the solutions were more dilute, indicating that the oxide layer is removed more rapidly than it is formed. This also can be noted in the polarograms obtained by Kolthoff and Tanaka (*4*): Reduction of the oxide layer on a preoxidized electrode gave cathodic dissolution currents which decayed rapidly to zero during the recording of the polarogram. The anodic currents corresponding to the formation of the oxide layer did not decay appreciably during the recording of the polarogram.

In potentiometric redox titrations the drifting of potential would be most noticeable in the vicinity of the equivalence point, as the concentration of excess reducing or oxidizing agent is very low.

Titration Curves. The effect of electrode oxidation on the shape of titration curves was studied for dichromate and cerate titrations of ferrous sulfate. In the forward titration 25 ml. of 0.1 and 0.01*N* ferrous sulfate solutions were titrated with 0.1 and 0.01*N* solutions of potassium dichromate and hexanitratoammonium cerate. All solutions were 1*N* in sulfuric acid. Before the start of the forward titration the electrode was prereduced in 0.1*N* ferrous sulfate for 10 minutes. Potentials were recorded during the course of the titration. A magnetic stirrer was used to ensure rapid mixing of the solutions. After the end point was passed, the titration was stopped and the electrode was kept in the titrated solution for 5 minutes. A back-titration with ferrous sulfate then was recorded. The results of these titrations are shown in Figure 4. The plots of the back-titrations have been corrected for the small difference in normalities of the ferrous solution and the oxidizing agent used in the titration. The titrations are reproducible to 0.02 ml.

A hysteresis effect is noted in comparing the forward- and back-titrations. During the forward-titration, the measured electrode potential lags behind the equilibrium potential as the oxide layer

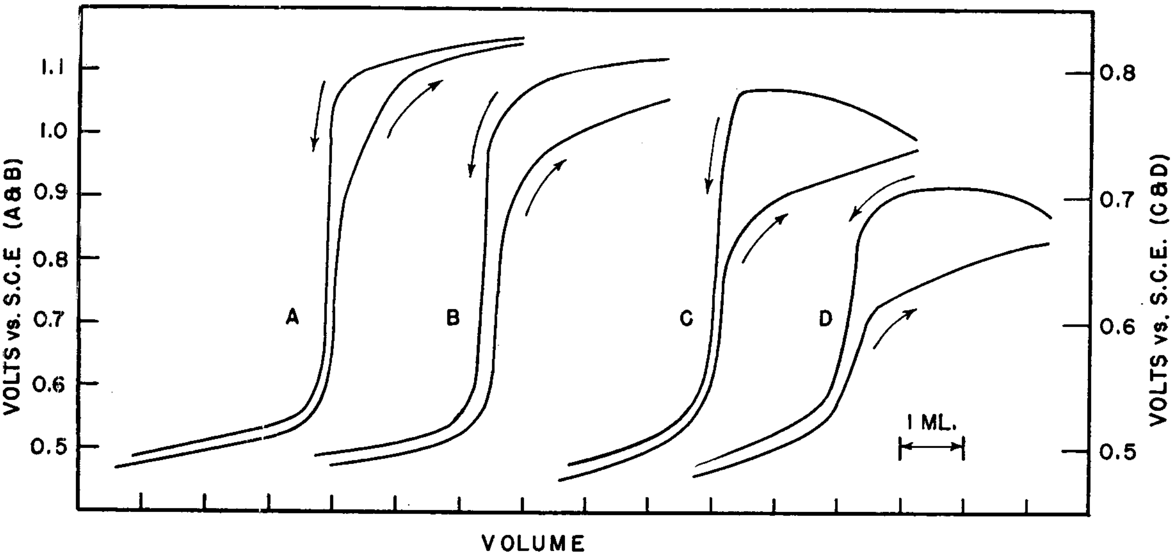

Figure 4. Titrations of ferrous sulfate with dichromate and cerate

A. 0.1*N* Fe(II) with 0.1*N* cerate *C.* 0.1*N* Fe(II) with 0.1*N* dichromate
B. 0.01*N* Fe(II) with 0.01*N* cerate *D.* 0.01*N* Fe(II) with 0.01*N* dichromate

is slowly formed, displacing the inflection point of the curve to the right. As the electrode has been preoxidized on standing in the titrated solution, a similar lag in electrode potential is observed in the back-titration, displacing the inflection point to the left. The removal of the oxide layer is a more rapid process than its formation, however, and the shape of the back-titration curve more closely approaches the theoretical curve calculated from the Nernst equation. The net result is a significant difference in inflection point volumes for the forward- and back-titration curves. This difference amounts to 0.20 ml. when 0.01*N* solutions are used and 0.10 ml. when 0.1*N* solutions are used for both the cerate and dichromate titrations. The greater discrepancy with the 0.01*N* solutions is expected, since the electrode surface is oxidized or reduced more slowly in dilute solutions. The effect would be even greater at higher delivery rates of the titrating agent.

The rise in potential observed in the back-titrations of dichromate solutions with ferrous sulfate has been discussed by Hendrixson (*3*) and Forbes and Bartlett (*1*).

As two different oxidizing agents produce essentially the same end-point error, these data indicate that many difficulties associated with potentiometric redox titrations are due to the oxidation or reduction of the surface of the platinum electrode. The titration curves show that extreme care should be taken in automatic potentiometric titrations in which the oxidizing agent is added until a certain potential is reached.

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RECEIVED for review November 25, 1955. Accepted January 17, 1956.

CRYSTALLOGRAPHIC DATA

III. Neodymium Sesquioxide, Nd_2O_3, Form *A*

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THREE polymorphic modifications of the rare-earth sesquioxides have been recognized, designated as *A*, *B*, and *C* (*1*). In the present study anhedral crystals of neodymium sesquioxide form *A* were prepared by fusing neodymium sesquioxide form *C* in an oxyacetylene flame (3000° to 3500° C.). The latter was prepared by heating "neodymium ammonium nitrate CP" (supplied by A. D. Mackay, New York, N. Y.) at 815° ± 20° C. for 72 hours in an electric furnace.

On further heating of the *C* form at between 885° and 985° C. for over 40 hours a mixture of the *C* and *A* forms was produced, but none of the *B* form, although these temperatures are well within the range of stability of neodymium sesquioxide form *B* reported by Goldschmidt, Ulrich, and Barth (*1*). The writer's observations are in better accord with those of Iandelli (*2*), who reported no *B* form for neodymium sesquioxide.

The crystal structure of the *A* form of the rare-earth sesquioxides propounded by Zachariasen (*7*) was refuted by Pauling (*3*), whose structure has been generally accepted (*4*, *6*). Zachariasen (*7*) and Swanson, Fuyat, and Ugrinic (*5*) have given powder x-ray diffraction data. (Swanson and coworkers seem to have been unaware of Pauling's structure, for they mention only that of Zachariasen.)

CRYSTAL MORPHOLOGY

Only anhedral crystals were obtained.

System and Class. Trigonal, hexagonal-scalenohedral (according to Pauling's structure).

Cleavage. {0001} prominent.

X-RAY DIFFRACTION DATA

Diffraction Symbol. $\bar{3}mP$---. Pauling's structure belongs to space group $P\bar{3}m1$ (D^3_{3d}).

Cell Dimensions. $a_0 = 3.841$; $c_0 = 6.002$ A.; $c/a = 1.564$; cell volume 76.78 A^3. Zachariasen (*7*) gives $a_0 = 3.84$, $c_0 = 6.01$ *kX*. Swanson and coworkers (*5*) give $a_0 = 3.831$, $c_0 = 5.999$ A.

Formula Weights per Cell. 1.

Formula Weight. 336.54.

Density. 7.277 grams per cc. (calculated from author's cell dimensions; weight of unit atomic weight 1.6602×10^{-24} gram).

OPTICAL PROPERTIES

Uniaxial negative.

Refractive Indices (5893 A.). $n_O = 2.11$, $n_E = 2.10$; geometric mean 2.107. Molecular refraction 24.7 cc. (from author's calculated density).

Color. Faint blue, with absorption $O > E$.

Table I. Powder X-Ray Diffraction Pattern

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
001	6.009	...	..
100	3.326	3.30	3
002	3.005	2.99	3
101	2.910	2.90	10
102	2.230	2.22	3
003	2.003	...	..
110	1.920	1.916	4
111	1.829	...	..
103	1.716	1.714	5
200	1.663	...	..
112	1.618	1.616	4
201	1.603	1.598	3
004	1.502	1.502	1
202	1.455	1.453	2
113	1.386	...	..
104	1.369	1.367	1
203	1.279	1.278	2
210	1.257	...	..
211	1.230	1.229	2
005	1.202	...	..
114	1.183	1.183	2
212	1.160	1.156	1
105	1.130	1.131	2
204	1.115	...	..
300	1.109	1.107	1
301	1.090	...	..
213	1.065	1.064	2
302	1.040	1.041	1
115	1.019	...	..
006	1.002	...	..
205	0.974	0.974	2
303	0.970	...	..
214	0.964	...	..
220	0.960	0.960	2
106	0.959		
221	0.948	...	..
310	0.923	...	..
222	0.915	0.914	1
311	0.912		
304	0.892	0.890	1
116	0.888		
312	0.882	...	..
215	0.869	0.868	<1
223	0.866		
007	0.858	...	..
206			
313	0.838	0.838	<1
400	0.832	0.832	<1
107	0.831		
401	0.824	...	..
305	0.815	...	..
224	0.809	...	..
402	0.801	...	..
314	0.786	...	..
117	0.784	...	..
216	0.783	...	..

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.

[b] Relative peak intensities above background from densitometer measurements.

Table II. Absorption Spectrum[a]

Ordinary Vibration Direction		Extraordinary Vibration Direction	
Wave length, mμ	Description	Wave length, mμ	Description
440	Strong, wide	440	Strong, medium
		441	Weak, narrow
444	Weak, medium		
		448	Medium, medium
		452	Weak, wide
469	Weak, narrow	469	Medium, narrow
471	Weak, narrow	471	Weak, narrow
477	Medium, wide	478	Medium, medium
479	Weak, narrow	480	Weak, medium
482	Weak, narrow		
484	Weak, narrow	485	Strong, narrow
487	Weak, narrow	487	Medium, narrow
		493	Strong, medium
496	Strong, medium	496	Weak, medium
512	Weak, narrow	510	Weak, wide
520	Medium, wide	519	Strong, medium
522	Weak, narrow	520	Weak, narrow
		525	Medium, medium
		533	Medium, medium
535	Strong, medium	536	Strong, medium
538	Strong, narrow	538	Medium, narrow
540	Strong, narrow	540	Medium, narrow
542	Weak, narrow		
545	Medium, narrow	544	Strong, narrow
		548	Weak, narrow
		551	Medium, narrow
581	Weak, narrow		
582	Strong, narrow	583	Medium, medium
588	Medium, wide	588	Strong, medium
		591	Medium, narrow
596	Medium, wide}	595–602	Medium
600	Medium, wide}		
607	Weak, narrow	605	Medium, medium
609	Strong, medium	609	Weak, medium
613	Strong, medium	613	Weak, medium
617	Strong, medium	617	Weak, medium
		630	Weak, narrow
		631	Weak, narrow
		639	Weak, narrow

[a] Band maxima in millimicrons, relative intensities, and relative widths of bands as viewed with Zeiss prism microspectrometer eyepiece.

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Work done under the auspices of the Atomic Energy Commission.

112. Samarium Sesquioxide, Sm_2O_3, Form *B*

R. M. DOUGLASS and EUGENE STARITZKY, The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

Anhedral crystals of samarium sesquioxide form *B* were prepared by Irving R. Tannenbaum of this laboratory by fusing samarium sesquioxide in an oxyacetylene flame (3000° to 3500° C). Form *B* has been recognized as one of the three polymorphic modifications of the rare-earth sesquioxides by Goldschmidt, Ulrich, and Barth (*1*), who described it as pseudotrigonal, orthorhombic or monoclinic. No other crystallographic data on this form have been published.

Crystal Morphology

The preparation examined consisted of coarse-grained aggregates of anhedral crystals.

X-Ray Diffraction Data

Diffraction Symbol. $2/mC\text{-}/\text{-}$, embracing space groups $C2/m$ (C^3_{2h}), $C2$ (C^3_2), and Cm (C^3_s).

Cell Dimensions (from calibrated Weissenberg and precession photographs). $a_0 = 14.17_7 \pm 0.01$ A.; $b_0 = 3.63_3 \pm 0.01$ A.; $c_0 = 8.84_7 \pm 0.01$ A.; $\beta = 99.96 \pm 0.03°$; $a:b:c = 3.90:1:2.44$; cell volume 448.8 A.^{3}

Formula Weights per Cell. 6.

Formula Weight. 348.86.

Density. 7.74 grams per cc. (calculated; weight of unit atomic weight 1.6602×10^{-24} gram); 7.68 (mean of densities of two small fused beads measured with Berman balance).

Optical Properties

Refractive Indices (5893 A.). $n_X = 2.070 \pm 0.005$, $n_Y = 2.093 \pm 0.005$, $n_Z = 2.095 \pm 0.005$; geometric mean 2.086. Molecular refraction 23.8 cc. (using calculated density).

Optic Orientation. $Z = b$.

Optic Axial Angle (5893 A.). $2V_X = 29^1/_2°$.

Color. Yellowish tan.

Absorption Spectrum (band maxima in millimicrons and relative intensities as viewed with Zeiss prism microspectrometric eyepiece). 468 (strong, wide), 472 (weak, wide), 482 (strong, narrow), 496 (very weak, narrow), 569 (weak, narrow).

Partial Powder X-Ray Diffraction Pattern[a]

hkl	*d*, A., Calcd.	*d*, A., Obsd.[b]	I/I_1[c]
111	3.212	3.185	3
401	3.063	3.040	3
$40\bar{2}$	2.988	2.980	10
003	2.905	2.890	4
310	2.864	2.843	5
$20\bar{3}$	2.863		
$11\bar{2}$	2.796	2.780	4
600	2.327	2.315	<1
$51\bar{1}$	2.220	2.208	4
$60\bar{2}$	2.218		
510	2.214		
601	2.157	2.149	4
$31\bar{3}$			
313	1.939	1.931	4
114	1.817	1.807	2
020			
021	1.778	1.764	3
$80\bar{1}$	1.771		
$20\bar{5}$	1.764		
404	1.719	1.711	4
$71\bar{2}$	1.715		
603	1.680	1.674	3
$40\bar{5}$			
022	1.677		
$51\bar{4}$	1.672	1.666	3
711	1.666		
$11\bar{5}$	1.589	1.586	2
421	1.562	1.553	3
$42\bar{2}$	1.552		
$80\bar{4}$	1.494	1.493	2

[a] Correlation of calculated and observed lines based on consideration of intensities of spots appearing on single-crystal photographs in addition to comparison of *d* spacings.

[b] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(\text{Cu}K\alpha) = 1.5418$ A.

[c] Relative peak intensities above background from densitometer measurements.

LITERATURE CITED

(1) Goldschmidt, V. M., Ulrich, F., Barth, T., *Skrifter Norske Videnskaps-Akad. Oslo, I, Mat.-Naturv. Kl.* 1925, No. 5, 5–24.

Work done under the auspices of the Atomic Energy Commission.

113. Indium Sesquioxide, In_2O_3

EUGENE STARITZKY, The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

INDIUM sesquioxide was prepared by E. J. Huber, of this laboratory, by ignition of indium metal in oxygen.

CRYSTAL MORPHOLOGY

System and Class. Isometric, diploidal.

Habit. Preparations examined consisted of anhedral grains and granular aggregates.

X-RAY DIFFRACTION DATA

The structure of indium sesquioxide has been determined by Zachariasen to be of the Tl_2O_3 type. Space group $Ia3$ (T_h^7).

Cell Dimensions. $a_0 = 10.1056 \pm 0.0010$ A.; cell volume 1032 A.^{3} Zachariasen (*1*) reported $a_0 = 10.14 \pm 0.03$ A.

Formula Weights per Cell. 16 (*1*).

Formula Weight. 277.52.

Density. 7.143 grams per cc. (x-ray).

OPTICAL PROPERTIES

Isotropic.

Refractive Index (5893 A.). 2.08. Molecular refraction 20.4 cc.

Colorless.

X-Ray Diffraction Pattern of In_2O_3

$h^2 + k^2 + l^2$	d, A., Calcd.	d, A., Obsd.[a]	I/I_1[b]
2	7.1457	...	..
4	5.0528	...	..
6	4.1256	4.11	12
8	3.5729	...	..
10	3.1957	...	..
12	2.9172	2.91	100
14	2.7008	2.69	2
16	2.5264	2.51	24
18	2.3819	2.37	6
20	2.2597	2.25	2
22	2.1545	2.142	7
24	2.0628	...	..
26	1.9819	1.973	13
30	1.8450	1.843	4
32	1.7864	1.780	49
34	1.7331	1.729	4
36	1.6843	1.677	4
38	1.6393	1.634	7
40	1.5978	1.593	4
42	1.5593	1.552	8
44	1.5235	1.519	42
46	1.4900	1.486	10
48	1.4586	1.456	10
50	1.42915	1.425	4
52	1.40139	1.398	3
54	1.37520	1.371	4
56	1.35042	1.350	2
58	1.32693	...	..
62	1.28341	1.281	6
64	1.26320	1.260	6
66	1.24391	1.242	7
68	1.22548	1.225	3
70	1.20785	1.205	2
72	1.19096	1.189	2
74	1.17475	1.175	6
76	1.15919	1.158	14
78	1.14423	...	..
80	1.12984	1.129	9
82	1.11598	1.115	8
84	1.10261	1.103	2
86	1.08971	1.088	5
88	1.07726	...	..
90	1.06522	1.065	5
94	1.04231	1.042	4
96	1.03140	1.031	9
98	1.02082	1.021	4
100	1.01056	1.009	2
102	1.00060	...	..
104	0.99094	0.9899	7
106	0.98154	0.9806	2
108	0.97241	0.9720	8
110	0.96353	0.9624	6
114	0.94648	0.9457	8
116	0.93828	0.9376	8
118	0.93030	0.9292	5
120	0.92251	0.9719	7
122	0.91492	0.9144	5
126	0.90028	0.9003	6
128	0.89322	0.8931	2
130	0.88632	...	..
132	0.87958	0.8792	3
134	0.87299	0.8725	3
136	0.86655	0.8666	2
138	0.86025	0.8602	2
140	0.85408	0.85384	6
142	0.84804	0.84096	2
144	0.84213	0.84184	3
146	0.83635	0.83606	5
148	0.83067	0.83061	2
150	0.82512	0.82511	2
152	0.81967	0.81964	4
154	0.81433	0.81407	3
158	0.80396	0.80377	4
160	0.79892	0.79882	4
162	0.79397	0.79393	2
164	0.78911	0.78906	8
166	0.78435	0.78439	2
168	0.77966	0.77959	5

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.; $\lambda(CuK\alpha_1) = 1.54050$ A.; $\lambda(CuK\alpha_2) = 1.54434$ A.

[b] Relative peak intensities above background from densitometer measurements.

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WORK done under the auspices of the Atomic Energy Commission.

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114. Zirconium Disulfate Tetrahydrate, $Zr(SO_4)_2.4H_2O$

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ZIRCONIUM disulfate tetrahydrate was prepared by allowing a solution of zirconium sulfate in dilute sulfuric acid to evaporate at room temperature or on the steam bath.

CRYSTAL MORPHOLOGY

System and Class. Orthorhombic, dipyramidal.

Axial Elements. Measurements were consistent with axial ratios determined by Weibull (*1*), $a:b:c = 0.6326:1:1.3350$. Matrix (030/001/100) transforms these to $a:b:c = 2.247:1:0.4739$, corresponding to the edges of the unit cell.

Habit. Crystals grown from hot solutions were generally tabular {010}, bounded by {100} and {311}. Equant bipyramids {311} were formed in solutions at room temperature.

Polar Angles. (311) ∧ ($3\bar{1}1$) = 43° 39′; (311) ∧ ($\bar{3}11$) = 59° 31′.

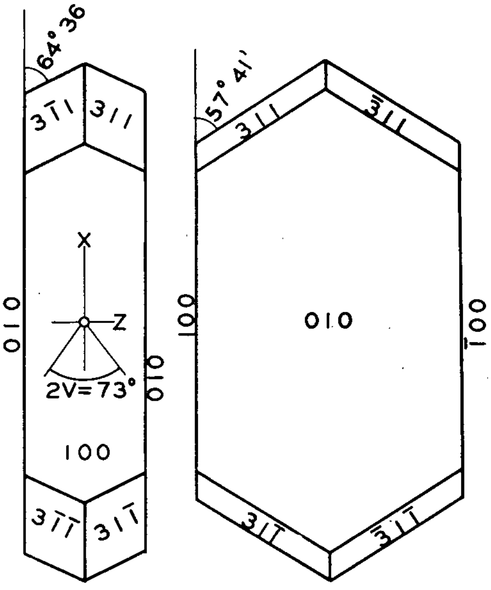

Figure 1. Crystal of zirconium disulfate tetrahydrate

Orthographic projections on (100) and (010)

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[1] Present address, Bridgeport Brass Co., Bridgeport, Conn.

Partial Powder X-Ray Diffraction Pattern of $Zr(SO_4)_2.4H_2O$

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
400	6.527	6.49	45
220	5.308	5.30	5
111	4.925	4.90	25
311	4.345	4.32	100
511	3.617	...	...
620	3.483	3.47	30
800	3.264	3.24	5
131	3.155	3.15	5
711	2.993	2.98	75
331	2.985		
040	2.905	2.91	15
202	2.719	2.71	5
531	2.715		
440	2.654	...	...
911	2.511	2.50	10
022	2.508		
222	2.463	...	...
731	2.419	2.408	20
10.2.0	2.382	2.373	10
602	2.343	2.332	35
422	2.341		
12.0.0	2.176	2.164	5
622	2.173		
840	2.170		
11.1.1	2.146	2.134	30
931	2.143		
151	2.137		
351	2.082	2.080	5
822	1.988	1.980	25
242	1.985		
551	1.984		
442	1.920	1.916	<5
260	1.916	1.894	15
10.0.2	1.903		
11.3.1	1.902		
13.1.1	1.865	1.855	5
751	1.859		
113	1.826	1.818	10
642	1.824		

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.
[b] Relative peak intensities above background from densitometer measurements.

X-RAY DIFFRACTION DATA

Space Group. *Fddd* (D_{2h}^{24}).

Cell Dimensions. $a_0 = 26.11$ A.; $b_0 = 11.62$ A.; $c_0 = 5.56$ A.; $a_0:b_0:c_0 = 2.247:1:0.478$; cell volume 1687 A^3.

Formula Weights per Cell. 8.

Formula Weight. 355.42.

Density. 2.80 grams per cc. (x-ray); 2.802 (flotation).

OPTICAL PROPERTIES

Refractive Indices (5893 A.). $n_X = 1.614$, $n_Y = 1.655$, $n_Z = 1.678$; geometric mean 1.6488. Molecular refraction (based on measured density) 46.2 cc.

Optic Orientation. $X = c$; $Y = a$; $Z = b$.

Optic Axial Angle (5893 A.). $2V_X = 73°$ with moderate dispersion $r < v$.

Colorless.

THERMAL DATA. Decomposition begins at 192° C. by growth of fine-grained parallel-fibrous aggregates along crystallographic planes. These eventually replace the crystal.

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(1) Weibull, M., *Ber. deut. chem. Ges.* 20, 1394–6 (1887).

WORK done under the auspices of the Atomic Energy Commission.

115. Ammonium Neodymium Disulfate Tetrahydrate, $NH_4Nd(SO_4)_2.4H_2O$

EUGENE STARITZKY and DON T. CROMER

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AMMONIUM neodymium disulfate was prepared by mixing aqueous or dilute sulfuric acid solutions of component sulfates in stoichiometric ratio.

CRYSTAL MORPHOLOGY

System and Class. Monoclinic, prismatic.

Axial Elements. $a : b : c = 0.350 : 1 : 0.465$; $\beta = 96.2°$ (derived from unit cell dimensions). For the isomorphous $NH_4Ce(SO_4)_2.4H_2O$ Kraus (*1*) found (after halving the *c*-axis to conform to the dimensions of the smallest unit cell) $a : b : c = 0.3598 : 1 : 0.4670$; $\beta = 97° 15'$.

Crystal Habit. Equant {110}, {021} with subordinate {011}. The clinodome faces commonly striated [100]. Cleavage {010}.

X-RAY DIFFRACTION DATA

Space Group. $P2_1/c$ (C_{2h}^5).

Cell Dimensions. $a_0 = 6.63$ A.; $b_0 = 18.94$ A.; $c_0 = 8.80$ A.; $\beta = 96.2°$; cell volume 1099 A^3

Formula Weights per Cell. 4.

Formula Weight. 426.51.

Density. 2.58 grams per cc. (x-ray); 2.584 (flotation).

OPTICAL PROPERTIES

Refractive Indices (5893 A.). $n_X = 1.531$, $n_Y = 1.540$, $n_Z = 1.549$; geometric mean 1.540. Molecular refraction 51.8 cc. (based on the measured density).

Optic Orientation. $Z = b$; $X \wedge c = 74°$.

Optic Axial Angle. $2V_X = 89°$ with weak dispersion $r > v$.

Color. Pale lavender without perceptible pleochroism.

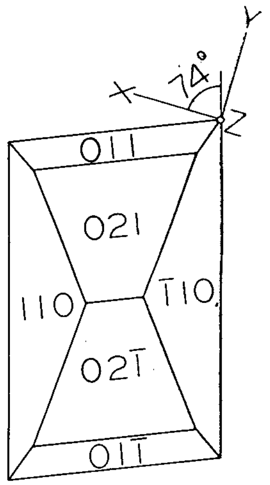

Figure 1. Crystal of ammonium neodymium disulfate tetrahydrate

Orthographic projection parallel to *b*

Partial Powder X-Ray Diffraction Pattern of Ammonium Neodymium Sulfate Tetrahydrate

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
020	9.47	...	...
011	7.94	7.93	<5
100	6.59	6.54	10
021	6.43	6.38	100
110	6.22	6.18	10
120	5.41	5.39	5
$\bar{1}11$	5.34	...	...
031	5.12	...	...
111	4.84	...	...
$\bar{1}21$	4.80	4.78	15
040	4.74	4.75	10
130	4.56	...	...
121	4.43	...	...
002	4.37	4.37	25
012	4.26	4.24	15
$\bar{1}31$	4.17	4.17	10
041	4.16		
022	3.97	3.95	10
131	3.92	...	...
140	3.85	3.85	10
$\bar{1}02$	3.84	...	...
$\bar{1}12$	3.76	...	...
$\bar{1}41$	3.61		
032	3.60	3.57	5
$\bar{1}22$	3.56		
102	3.48	3.47	<5
051	3.48		
141	3.44		
112	3.42	3.38	5
200	3.296		
150	3.284	3.28	10
$\bar{1}32$	3.281		
122	3.263	...	...
210	3.247	3.23	20
042	3.213	3.21	10
060	3.157	3.16	10
$\bar{2}11$	3.155		

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(\mathrm{Cu}K\alpha) = 1.5418$ A.

[b] Relative peak intensities above background from densitometer measurements.

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(1) Groth, P., "Chemische Krystallographie," Bd. II, p. 561, Engelmann, Leipzig, 1908.

WORK done under the auspices of the Atomic Energy Commission.

116. Dineodymium Trisulfate Pentahydrate, $Nd_2(SO_4)_3.5H_2O$

EUGENE STARITZKY and DON T. CROMER

The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

DINEODYMIUM trisulfate pentahydrate crystallizes from strong sulfuric acid solutions of neodymium sulfate.

CRYSTAL MORPHOLOGY

System and Class. Monoclinic, prismatic.

Axial Elements. $a : b : c = 1.639 : 1 : 1.066$; $\beta = 120.3°$ (derived from unit cell dimensions). For the isomorphous $Ce_2(SO_4)_3.5H_2O$, Kraus (*1*) determined $a : b : c = 1.4656 : 1 : 1.1264$; β 102°40′. On transformation by matrix ($\bar{1}0\bar{1}/0\bar{1}0/001$), to conform to the orientation of the unit cell, this becomes $a : b : c = 1.6410 : 1 : 1.1264$; $\beta = 119° 23'$.

Habit. {110} prisms terminated by {$\bar{1}11$}; {100} also often present.

X-RAY DIFFRACTION DATA

Diffraction Symbol. $12/m1C\text{-}c\text{-}$; observed development of crystal forms and failure to detect a piezoelectric effect make it probable that the space group is $C2/c$ (C^6_{2h}).

Cell Dimensions. $a_0 = 15.75$ A.; $b_0 = 9.61$ A.; $c_0 = 10.24$ A.; $\beta = 120.3°$; cell volume 1338 A^3

Formula Weights per Cell. 4.

Formula Weight. 666.82.

Density. 3.31 grams per cc. (x-ray); 3.309 (flotation).

Partial Powder X-Ray Diffraction Pattern of Dineodymium Trisulfate Pentahydrate

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
110	7.85	7.76	100
$\bar{1}11$	6.96	6.88	20
200	6.80	...	...
111	5.17	5.14	30
$\bar{2}02$	5.05	5.01	15
020	4.81	4.76	5
$\bar{3}11$	4.61	4.56	10
$\bar{1}12$	4.45	4.42	15
002	4.42		
021	4.22	4.19	10
310	4.10	...	...
$\bar{3}12$	4.07	4.05	5
$\bar{2}21$	4.05	4.02	15
220	3.92	...	...
$\bar{4}02$	3.76	3.74	5
$\bar{2}22$	3.48	3.46	10
112	3.45		
400	3.40	3.38	20
022	3.25	3.24	40
221	3.25		
311	3.20	3.18	10
$\bar{3}13$	3.18	3.16	50
130	3.12	3.10	25
$\bar{1}13$	3.07	3.07	5
202	3.07		
$\bar{1}31$	3.05		
$\bar{4}21$	3.03	...	...
$\bar{5}12$	2.97	2.95	10
$\bar{4}22$	2.96		
$\bar{5}11$	2.93	...	...
131	2.84	2.83	15

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(\mathrm{Cu}K\alpha) = 1.5418$ A.

[b] Relative peak intensities above background from densitometer measurements.

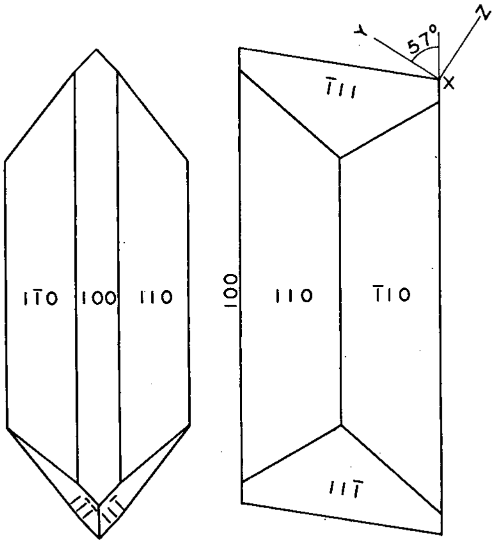

Figure 1. Crystal of dineodymium trisulfate pentahydrate

Orthographic projections on (100) and parallel to *b*

OPTICAL PROPERTIES

Refractive Indices (5893 A.). $n_X = 1.582$, $n_Y = 1.600$, $n_Z = 1.608$; geometric mean 1.5966. Molecular refraction 68.6 cc.

Optic Orientation. $X = b$; $Y\!Ac = 57°$.

Optic Axial Angle (5893 A.). $2V_x = 66°$ with weak dispersion $r < v$.

Color. Lavender, without perceptible pleochroism.

THERMAL DATA. Dehydration begins at 107° C., evidenced by irregular extinctions, clouding, and breaking up of crystals.

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(1) Groth, P., "Chemische Krystallographie," Bd. II, p. 450, Engelmann, Leipzig, 1908.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, 3140 South Michigan Ave., Chicago 16, Ill. Work done under the auspices of the Atomic Energy Commission.

SCIENTIFIC COMMUNICATION

Gravimetric Determination of Palladium(II) and Mercury(II) as Bisethylenediaminepalladium(II) Tetraiodomercurate(II)

SIR: We have observed that milligram quantities of mercury(II) or palladium(II) may be precipitated quantitatively as bisethylenediaminepalladium(II) tetraiodomercurate(II), $[Pd(en)_2]HgI_4$. This precipitation is not influenced adversely by excess of the precipitating reagent or most common anions and the precipitate is easily filtered and dried to constant weight. This salt is substantially insoluble in the pH range 6 to 8 and is only slowly attacked by hot concentrated nitric acid. These properties, together with the high molecular weight (935) and favorable gravimetric factor, suggest the use of this precipitation for the gravimetric determination of either mercury(II) or palladium(II).

We have established the applicability of this method to our own rather specific needs and have obtained uniformly satisfactory results. Limited studies on interferences indicated that precipitation with the tetraiodomercurate(II) ion may prove useful for the quantitative precipitation of complexes of other transitional metal ions.

Reagents. Bisethylenediaminepalladium(II) bromide was prepared by fuming palladium(II) nitrate or chloride to dryness several times with hydrobromic acid, heating this product with a slight excess of aqueous ethylenediamine, and precipitating the salt by the addition of a 10-fold volume of ethyl alcohol. The product was filtered, washed with absolute ethyl alcohol, and dried in vacuo at room temperature. Analysis. Calculated for $[Pd(en)_2]Br_2$: Pd, 27.6. Found (by the dimethylglyoxime method): Pd, 27.6. A 0.8052-gram sample of this product was dissolved in water to provide 100.0 ml. of solution, which was used as the standard palladium solution. Somewhat more concentrated (ca. 0.05*M*) solutions of this compound were used as a precipitation reagent.

The standard solution of potassium tetraiodomercurate(II) contained 0.3239 gram of mercury(II) iodide and 1.0 gram of potassium iodide in 100.0 ml. of solution; solutions ca. 0.09*M* with respect to tetraidomercurate ion were used as precipitation reagents.

Solutions of the following compounds were used in testing for cationic interferences: bisethylenediamineplatinum(II) bromide (*4*), bisethylenediaminegold(III) bromide (*1*), tetrammineplatinum(II) bromide (*6*), chloropentamminechromium(III) chloride (*5*), bisethylenediaminecopper(II) chloride (*3*), and trisethylenediaminenickel(II) chloride (*2*).

Determination of Palladium. Aliquots of the standard solution of bisethylenediaminepalladium(II) bromide solution were adjusted to pH 6 to 8 and treated dropwise with potassium tetraiodomercurate(II) solution until no further precipitation was evident. The precipitate was allowed to coagulate for about 10 minutes, filtered on a tared fritted-glass crucible, washed with distilled water, and dried to constant weight at 115°. Thus, 1.0-, 3.0-, 5.0-, and 10.0-ml. aliquots containing, respectively, 2.22, 6.66, 11.10, and 22.20 mg. of palladium were found by this procedure to contain 2.18, 6.64, 11.11, and 22.24 mg., respectively. The composition of the salt precipitated was further confirmed by ignition of a weighed sample to palladium(II) oxide. Found: Pd, 11.7. Calculated for $[Pd(en)_2]HgI_4$: Pd, 11.4.

Table I. X-Ray Diffraction Data for Bisethylenediaminepalladium(II) Tetraiodomercurate(II)[a]

d, *A*.	I/I_0	*d*, *A*.	I/I_0	*d*, *A*.	I/I_0
7.80	0.9	2.56	0.2	1.69	0.1
6.43	0.7	2.47	0.6	1.66	0.4
4.94	0.1	2.24	0.4	1.61	0.3
4.63	0.8	2.18	0.2	1.51	0.4
4.25	0.7	2.14	0.2	1.47	0.3
3.99	1.0	2.02	0.5	1.45	0.3
3.73	0.7	1.96	0.4	1.42	0.1
3.48	1.0	1.90	0.3	1.38	0.1
3.23	0.1	1.86	0.2	1.34	0.1
3.08	0.7	1.83	0.2	1.28	0.2
2.88	0.1	1.80	0.2	1.24	0.1
2.77	0.8	1.74	0.4	1.23	0.1
2.68	0.2			1.13	0.1

[a] From pattern obtained using Cu*K*α radiation, 15-ma. filament current, 35-kv. tube voltage, and exposure time of 6 hours.

Apparently the only precaution necessary is to ensure that the precipitate does not dry on the walls of the beaker, from which the precipitate is difficult to remove. Before bisethylenediaminepalladium(II) tetraiodomercurate(II) is dried, it exhibits appreciable solubility in ethyl alcohol and acetone, but is substantially insoluble in these solvents after thorough drying at 115°. X-ray diffraction data for this compound are given in Table I.

Determination of Mercury. Aliquots (1.0, 5.0, and 10.0 ml.) of the standard potassium tetraiodomercurate(II) solution were adjusted to pH 6 to 8 and treated with a slight excess of bisethylenediaminepalladium(II) bromide or iodide solution; the precipitates were treated as described above. These three aliquots contained 1.43, 7.15, and 14.3 mg. of mercury, as compared with 1.30, 7.06, and 14.2 mg. found, respectively.

Interferences. Tests for possible interferences by common anions were carried out as follows:

To 10 ml. of water was added a quantity of hydrochloric acid such that the solution finally analyzed for palladium was ca. 0.05*M* with respect to chloride ion. Bisethylenediaminepalladium(II) iodide was dissolved in this solution, the pH was adjusted to 7 to 8, and an aliquot was analyzed for palladium by the dimethylglyoxime method. Found: Pd, 18.3 mg.

Another aliquot was analyzed by precipitation of bisethylenediaminepalladium(II) tetraiodomercurate(II) as described above (found: Pd, 18.4 mg.), and the palladium content calculated from the data obtained by igniting this precipitate to palladium(II) oxide was 18.4 mg.

In strictly analogous experiments, the weights of palladium found in the presence of the following anions are: bromide 18.4, iodide 18.3, nitrate 18.5, sulfate 18.4, and perchlorate 18.5. That the precipitates obtained in these experiments did not contain anions other than the tetraiodomercurate(II) ion was confirmed by means of x-ray diffraction patterns. Accordingly, it may be concluded that the common anions do not interfere with this precipitation.

Similar tests for interferences by transitional metal cations show that bisethylenediamineplatinum(II), tetrammineplatinum(II), bisethylenediaminegold(III), chloropentamminechromium(III), bisethylenediaminecopper(II), and trisethylenediaminenickel(II) ions interfere and must therefore be removed prior to the precipitation of either palladium or mercury. Both of the platinum(II) complexes apparently precipitate as the ditriiodomercurate(II) salts, although under other conditions the tetraiodomercurates may also be obtained. Preliminary results also suggest that interferences by complexes of copper(II) and nickel(II) may be obviated by carrying out the precipitation in somewhat more acidic solutions.

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RECEIVED for review July 14, 1955. Accepted February 6, 1956. Work supported in part by the Atomic Energy Commission, Contract AT-(40-1)-1639.

Water Repellency of Silicone on Microburet Tips

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WHERE volumetric measurements are approximated to 0.001 ml. using the Koch microburet, rigid control of the size of the droplets is desirable. Occasionally the droplets may fail to hang freely and may tend to form by creeping upward, away from the orifice, owing to the hydrophilic nature of glass. In such instances suspicion may be directed to contamination or age of the standard solution or to a newly greased stopcock. The condition may be remedied by drying the end of the tip with cleansing tissue and immediately rubbing it with a silicone-treated tissue (Sight Savers), thus rendering the surface on the ground point of the tip hydrophobic. The silicone in the prepared tissues appears to be more effective than some other silicones.

After coating the dropping tip of a Koch microburet with silicone, individual volumes of about 0.0001 ml. can be delivered easily. This degree of control may be of some consequence in such volumetric procedures as calibrating micro volumetric glassware or determining the volume of a small, irregularly shaped, biological specimen. The application of a silicone coating to the tips of capillary burets and capillary pipets may prove useful in quantitative ultramicroanalysis.

Filter System for Radioactive Exhaust from Flame Spectrophotometer

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FLAME spectrophotometric analysis of radioactive solutions is hazardous because the radioactivity is dispersed in the vapors of the flame and in the exhaust gases. Provision for removing this radioactivity is not usually made in laboratory fume hoods or on flame spectrophotometers. An effective and economical filter system for removing the radioactivity has been built which uses a special filter (*4*) in an auxiliary exhaust system attached to the burner housing of the spectrophotometer. Other filter media, such as those described by Stafford (*3*) and by Smith (*2*), should prove equally satisfactory for this application.

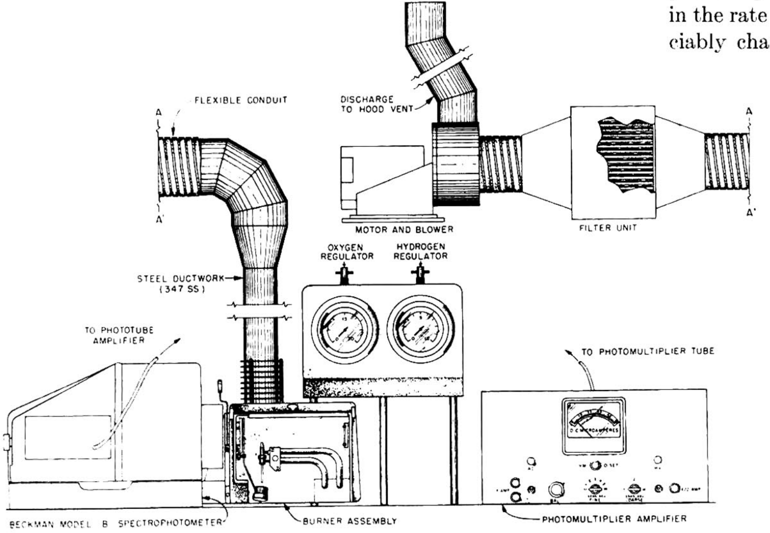

Figure 1. Exhaust filter system for flame spectrophotometer

The filter system built for use on the Beckman Model B flame spectrophotometer is shown in Figure 1. A 2.5-inch-diameter duct of 16-gage, Type 347 stainless steel is attached to the exhaust port of the burner housing. This duct extends vertically for 24 inches above the housing and then flares into a right-angled elbow, which is connected to the filter unit by a 4-inch-diameter flexible conduit of galvanized iron. This system of ducts acts as a heat exchanger for the hot exhaust gases, and is designed to facilitate replacement of the filter unit. By means of another section of flexible conduit, the filter unit is attached to a 2-inch-diameter squirrel-cage blower (Buffalo Forge Co.) powered by a 0.10-hp. motor rated at 1500 to 3000 linear feet per minute of air flow. The filtered air is discharged into the fume hood system of the laboratory. The rate of air flow at the port of the burner housing was determined to be 1200 linear feet per minute. The auxiliary blower and the filter should be mounted on a shelf above the flame spectrophotometer at a minimum distance of 3 feet from it. This arrangement facilitates proper handling of radioactive solutions and permits the use of lead shields if necessary. The entire assembly fits conveniently inside the average hood space of 4 by 8 feet.

The most important feature of the filter system is the filter medium, which is a specially prepared asbestos-impregnated paper developed by Arthur D. Little, Inc. This paper is arranged in deep multiple pleats over fluted aluminum foil separators. The filter medium is mounted in a frame to form a compact unit 8 inches square and 4 inches deep. Tests (*1*) made on atmospheric dusts and on aerosols with average particle-size diameters of 0.3 to 0.5 microns indicate that the efficiency of the filter is as high as 99.999%. The tests were made at air flow rates equivalent to a static pressure differential of 1 inch of mercury and at temperatures up to 250° F.

Periodic examination of the duct system and filter unit has indicated that the filter efficiently removes the radioactivity from the air stream being discharged into the fume hood system of the laboratory. A typical radiation survey showed 1 milliroentgen per hour of radiation at contact along the exterior of the duct that leads from the burner housing to the filter unit; radiation of 8 to 13 milliroentgens per hour was detected at the filter unit. No radioactivity was detected inside the burner housing or along the duct that leads from the filter case to the hood exhaust duct.

The filter is replaced when the rate of air flow decreases to less than 800 linear feet per minute or when the radioactivity accumulated on the filter causes background radiation greater than 7.5 milliroentgens per hour at the position where the flame spectrophotometer is operated.

This filter system has been in use for approximately two years in conjunction with a Beckman Model B flame spectrophotometer, equipped with a photomultiplier tube and a sensitive external photoamplifier. Use of the system, which results in an increase in the rate of air flow through the burner housing, does not appreciably change the luminosity readings obtained with the flame spectrophotometer. However, new calibration curves should be prepared when the exhaust fan and filter unit are put into operation with the flame spectrophotometer.

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Rapid Automatic Scanning Device for Paper Electrophoresis and Chromatography

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During the course of investigation into the nature of allergenic materials, a technique was developed to attain rapid, automatic scanning of a large number of paper electrophoretic strips. One of the technical obstacles in the analysis of a large number of electrophoretic filter paper patterns is the tedious and time-consuming plotting of curves even with the use of specialized densitometers available. For this purpose an improved apparatus was designed as an attachment to the Model B Beckman spectrophotometer (*1, 3, 8, 11*). The Model B was used because both sensitivity and wave lengths could be easily adjusted to the requirements of the various stains used. Pretreatment of the paper strips was unnecessary regardless of the type of paper used although some workers obtain better results with oiled strips. The scanner attachment, Figure 1, consists of two film-type spools with an adjustable paper strip guide permitting the use of strips 1 inch wide or less. A direct drive rubber roller pulls the paper across the photo tube slit. The wind-up spool is attached to the drive roller by means of a slip clutch while the unwinding spool turns freely. A small synchronous motor turns the linear driving mechanism at a rate of 2 inches per minute. A manually operated calibrated drive can be used in place of the motor if desired. The entire procedure was made completely automatic by attaching a 1-ma. Esterline-Angus strip chart recorder to leads numbers 11 and 13 on the terminal strip in the Model B, Figure 2. An external 10,000-ohm potentiometer was used to correlate galvanometer reading with the chart pen writer. Gears (36 to 54 ratio) were obtained from the Esterline-Angus Co., Indianapolis, Ind., to correlate the chart recorder speed with that of the scanning attachment.

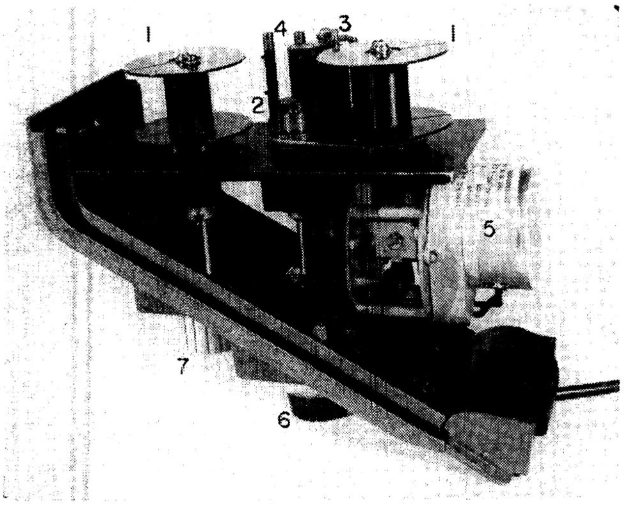

Figure 1. Scanning device

1. Spools
2. Slip clutch
3. Driving mechanism
4. Adjustable paper guide bars
5. Synchronous motor unit
6. Helipot manual turning control
7. Rewind control

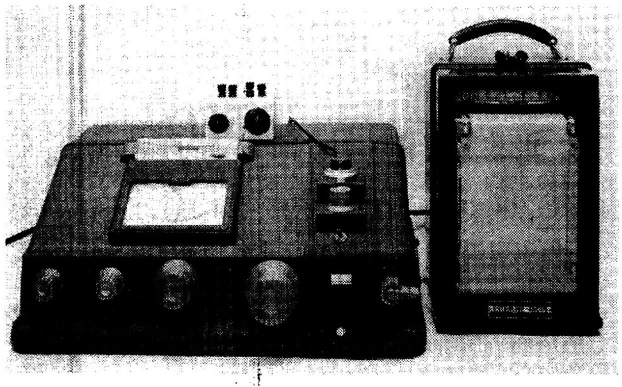

Figure 2. Apparatus with external variable resistance box, recorder, and spectrophotometer

The paper electrophoresis apparatus used was essentially that of Kunkel (*7*). The Durrum designed Spinco Model R paper electrophoresis unit was also used (Spinco Div., Beckman Instrument Corp., Palo Alto, Calif.). Following an electrophoretic run, the strips were removed and stained directly with bromophenol blue (*2*) or dried and stained with ninhydrin (*4, 9*).

A 1-inch-wide plastic framing trough was used to effect accurate joining of the strips in series with black plastic tape which appears as a total deflection on the chart recorder. Up to 10 strips were joined and then placed on the spools for scanning. A normal human serum standard, run concurrently, was scanned in the series to establish a base line. The range between 550 to 490 mμ gave a better resolution of bromophenol blue and ninhydrin patterns. The base line was determined by arbitrarily setting 100% transmittance at a point between the alpha 2 globulin and albumin in the human serum pattern and at a point between components obtained with other materials. The value of areas under the obtained curves were determined with a planimeter, rough visual comparison, or cutting out and weighing the comparative areas. Figure 3 illustrates the results obtained with the normal human serum pattern. The obviously low albumen value in Figure 3 is the result of certain variables in technique which can be manipulated at will (*6*). Although there are many shortcomings and inherent errors (*5, 10*), this method is well-suited for routine analysis. Paper chromatograms up to a width of 1 inch could be scanned with equal facility.

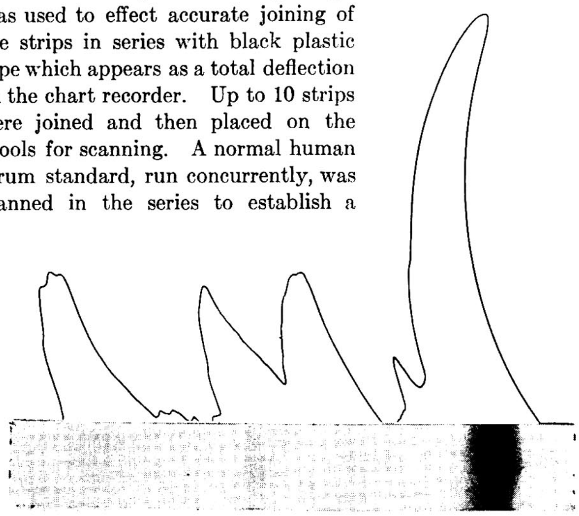

Figure 3. Schlieren pattern obtained with normal human serum

Vernal buffer, pH 8.6, 0.1 ionic strength, run 14 hours at room temperature in Durrum electrophoresis cell

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ANALYTICAL CHEMISTRY

PART II—April 1956

Review of Fundamental Developments in Analysis

Light Absorption Spectrometry

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THE literature pertaining to light absorption spectrometry for the period from August 1953 to August 1955 has been surveyed for this report. The reviewers have attempted to winnow these publications and to document those papers presenting significant contributions to the field. As in any such elimination and selection process, the judgment of the reviewers should not be expected to be inerrant. The general outline of the preceding reviews (*394, 395*), in which the subject matter was divided into three categories, has been followed. These three categories are: the chemical aspects of the system exhibiting characteristic light absorption, the physical aspects underlying the measurement of light absorption, and specific analytical applications.

CHEMISTRY

Although the mechanism of the reaction and the structure of the resulting product exhibiting characteristic light absorption may be unknown, this does not preclude the usefulness of the colorimetric method based upon such a reaction. Progress has been made in elucidating the structure of some of these light-absorbing systems and in understanding the stoichiometry and kinetics of the reactions involved. New reagents, or modifications in procedure, especially in respect to methods of separation and complexation, have resulted in improved sensitivity, reliability, and applicability of colorimetric methods. Although cognizant of the possibility for some overlapping, the recent advances related to the chemistry of the light-absorbing systems have been considered under the headings of reactions, structures, reagents, modifications in preliminary chemical treatment prior to color measurement, and methods of analytical separation.

Reactions. METALS. The kinds of reactions used are considered in terms of applications to metals, nonmetals, organic systems, and specific cases. The classical reaction of chromate and diphenylcarbazide has been thoroughly investigated by a variety of physicochemical methods (*69*). It was found that chromate and diphenylcarbazide, or chromate and carbazone, gave the same red-violet compound as was obtained with chromium(II) ions and carbazone. The chromium(II) ion and carbazone form an inner-complex compound. Carbadiazone and carbazone are products in the reduction of chromate with diphenylcarbazide, and carbadiazone is the reduction product between chromate and carbazone. This postulated mechanism indicates the formation of the enol form of the chromium(II)-carbazone complex.

Several reactions involving titanium(IV) have been studied. The reaction with ascorbic acid through the enediol group results in the maximum development of color at pH 4.5 (*91*). When the ascorbic acid–titanium(IV) ratio is high, 50 to 1, the color is proportional to the titanium concentration. At intermediate ratios, 10:1 to 1.25:1, the color is proportional to neither the titanium nor the ascorbic acid concentration. For low ratios, 1.25:1 to 0.125:1, the color is proportional to ascorbic acid. Hence, it was recommended that the buffer and titanium(IV) solutions be added to the ascorbic acid reagent. The reaction of chromotropic acid with titanium(IV) has been studied critically (*73*) and compared with the reactions in which bi- and trivalent phenols were used as reagents (*473*). Spectrophotometric evidence indicates polymeric species in concentrated solutions of peroxytitanic acid but only the monomer in dilute solutions (*522*). A titanium(IV) thiocyanate complex is reported to have been formed in an acetone-aqueous medium under carefully controlled conditions (*115*).

The reaction of vanadium(V) with potassium benzohydroxamate gives three different colors, depending upon acidity (*121*). The color is blue in strong acid solutions, red in weak acid solutions, and purple in ammonia solutions. Although of limited stability, the red complex is best for the colorimetric estimation of vanadium. In an indirect colorimetric method, vanadium(V) is reduced to vanadium(IV) by boiling with concentrated hydrochloric acid. Upon adding iron(III), the vanadium(IV) is oxidized to vanadium(V) with an equivalent amount of iron(II) being produced. The iron(II) is then determined with 1,10-phenanthroline (*269*).

The mechanisms of the thiocyanate reactions with molybdenum and tungsten have been elucidated (*116*). Reactions involving the colored complexes of palladium(II), rhodium(III), and platinum(II) have been investigated by Ayres (*15*).

In a new color reaction zinc reacts with *p*-aminophenol and potassium ferricyanide at pH 4 to 7 to give a red colored system which shows conformity to Beer's law (*253*).

Salicylamide reacts stepwise with uranyl ions in solutions of pH 6.6 to 7.2 to give $UO_2(C_6H_4OCONH_2)$, which has a yellow color conforming to Beer's law for 1 to 150 p.p.m. of uranium. The instability constant for the over-all reaction is given as pK = 11.37 (*96*).

The reactions of metallic ions and numerous organic reagents have been studied with the stability of the resultant complex being of interest as well as its characteristic light absorptivity. In the reaction of kojic acid, 2-hydroxymethyl-5-hydroxy-γ-pyrone, with a number of cations the formation constants of the metal kojates were determined (*79*). With benzenoid resonance being impossible, the stability of these complexes is related to the formation of a five-membered ring. 2-(*o*-Hydroxyphenol)-benzoazole and 2-(*o*-hydroxyphenyl)benzothiazoline reacts with a number of metal ions to give six-membered ring chelates (*98*). In general, the six-membered rings are less stable than five-membered rings. Iron(II) reacts with 1,2-cyclohexanedione-dioxime to give a complex in which the iron-reagent ratio is 1 to 2 (*31*). The reaction of iron(II) with dimethylglyoxime, using dithionate to stabilize the color of the complex, has been utilized in the colorimetric determination of iron (*256*). The reaction between nickel(II) and dimethylglyoxime in ammoniacal solutions in the presence of oxygen results in the formation of two complexes (*682*). The colored complex has a nickel-reagent ratio of 1 to 3, while the colorless complex has a 1 to 1 ratio. The iron(III) thiocyanate reaction has been examined critically (*350*).

Nonmetals. Utsumi (*638*) has determined chloride, thiosulfate, sulfide, iodide, and cyanide by means of indirect colorimetric methods using the iron(III) thiocyanate reaction. Basic lanthanum acetate reacts with iodide to give a yellow-green to blue-green color which is suitable for the estimation of acetate ion (*311*). As the result of a study of the reaction between aluminum ion and hematoxylin it was concluded that hematin, produced by the oxidation of hematoxylin, is the complexer and that fluoride ions destroy the complex through the formation of aluminum hydroxyfluoride (*372*). The reaction of hydroxylamine with iron(III) to form iron(III) hydroxylamate is suitable for the determination of hydroxylamine (*214*).

Organic Compounds. The mechanism of the Mohler reaction, which is used in the colorimetric estimation of benzoic acid and mononitro- and dinitrobenzoic acids (provided nitro groups are ortho or para to each other) has been elucidated. According to the proposed mechanism, 2,5-dinitrobenzoic acid is formed first by nitration and then reacts with hydroxylamine to give a quinoid structure (*122*). The reaction of imino acid with 1,2-naphthoquinone-4-sulfonate has been investigated (*631*). The Komarowsky reaction has been utilized in the spectrophotometric determination of 1,2-propylene glycol in ethylene glycol, cyclohexanol and cyclohexanone in cyclohexane, and isovaleraldehyde in succinic acid (*119*). The colorimetric determination of 2-methyl-8-quinolinol based on the diazonium reaction in basic solution has been suggested (*45*). It has been shown that the sensitivity of the formaldehyde-leucomethylene blue reaction is increased in the presence of ethanol, acetone, diethyl ketone, or methyl ethyl ketone (*56*). Certain mononitro aromatic compounds react with tetraethylammonium hydroxide in dimethylformamide to give stable colors suitable for colorimetric measurement (*508*). The reaction between blue tetrazolium and steroids was investigated with time for color development, temperature, and basicity as the main variables. Correlation between absorbance and the position of the structural function in the polycyclic nucleus was possible (*399*).

Special Reactions. A catalytic reaction in which mercury(II) catalyzes the formation of a violet complex between ferrocyanide and nitrosobenzene is applicable to the determination of mercury(II) ions in distilled water. The color, after a definite time, is proportional to the concentration of mercury(II) ions (*13*). The rapid rate for the reaction of iron(II) with 1,10-phenanthroline as compared with the rates of the reactions of this reagent with vanadium(IV), chromium(III), and nickel(II) has been used in a *spectrophotometric method for determining minute* traces of iron in these metals (*380*).

Structures. This section summarizes many of the significant advances which have been made in the study of metal-chelates with attention being focused on the formula of structures responsible for light absorption. The light absorption of metal complexes has been attributed to nonbonding electrons of the ligand, nonbonding electrons of the cation, and bonding electrons between the metal and ligand (*666*). The relationship between the intensity of the absorption band and the nature of the ligand has been investigated for the cobalt(III) complexes (*675*). The absorption spectra of the chelates formed by cobalt, nickel, copper, palladium, and iron with 8-quinolinol, salicylaldehyde, salicylaldoxime, salicylaldimine, and dimethyl- and diphenylglyoxime have been determined (*597*). The quantum mechanical theory of light absorption has been used in relating the color and constitution of complex ions (*227*). The anomalous bands due to chromophores and the relationship between stability of the complex and the shift in absorption spectra have been studied. In a study of the relationship between absorption spectrum and the structure of the iron(III) halide complexes, the bathochromic shift, which increased with the number of chloride ligands, was explained in terms of electron-transition bands (*560*). The fact that some iron(III) complexes, such as tris(1-nitroso-2-naphthol) iron(III), exhibit maximum absorptivity in the visible region has been attributed to the small energy difference between iron(II) and iron(III), which facilitates resonance of the organic radical (*596*). Factors involved in the formation of coordination compounds and the stability of these compounds have been outlined for the case of oxygen and nitrogen coordination (*642*). A discussion of factors affecting the stability of complex ions and methods of determining the stability of complexes has been given by Jonassen (*284*). The thioamide group is a reactive functional group for iron and bismuth because of tautomerization (*634*). The study of the effect of methyl and phenyl substitution in 2,2-biquinoline has shown an improvement in absorptivity with phenyl substitution. Thus, 4,4-diphenyl-2,2-biquinoline, giving a molar absorptivity of 9020, is the most efficient biquinoline (*592*). The stability of complexes of bivalent ions of several transition elements has been determined (*262*).

In acidic solution, copper(II) complexes are formed with dimethylglyoxime having the formulas, $CuHDm^+$ and $Cu(HDm)_2$. In basic solutions, intensely colored compounds believed to have formulas corresponding to CuDm and $Cu(Dm)_2^{--}$ are formed (*20*). The nature of the mixed complex of copper(II) with oxalate ion and ethylenediamine has been investigated spectrophotometrically (*133*). Using the bis(ethylenediamine)-copper(II) and the bis(oxalate)-cuprate(II) ions it has been found that the mixed complex predominates in the pH range of 6 to 6.5. The copper(II) and iron(III) chelates formed with citric acid were found to have a 1 to 1 ratio, and the equilibrium constants for intermediate species of ions were determined (*652*). The copper(I)–2,2′-biquinoline complex has a purple color in a 1 to 1 dimethylformamide-water medium which is suitable for the colorimetric determination of copper(II). The solvent functions as reducing agent in reducing copper(II) to copper(I) prior to chelation (*496*). A spectrophotometric study of copper halide complexes indicates that the copper(I) halides are usually contaminated to some extent by copper (II) halide (*330*). The existence of $CuCl^+$ in dilute chloride solutions (*425*) and the ionization constant of copper(II) sulfate (*426*) have been determined by spectrophotometric methods. Copper(II) forms two complexes with propylenediamine in aqueous solution, in which the dipositive ions contain one and two molecules of the amine (*221*). Evidence has been found for the formation of a copper(II) acetate complex in the presence of sulfate, perchlorate, or nitrate ions (*66*). Using triphenylmethylarsonium thiocyanate as a reagent, a stable copper complex having the formula,

$(R_4As)_2Cu(SCN)_4$, has been isolated (*143*). Ballhausen (*29*) has outlined the theory of the copper(II) spectra.

Cobalt(II) and dimethylglyoxime, in acetone medium and in the presence of air, form a complex having the formula of $HCo(HDm)_2Cl_2$. Cobalt(II) does not react with dimethylglyoxime in strongly acidic solutions. In slightly acidic or basic solutions a compound having the formula $Co(HDm)_2.8H_2O$ is formed (*21*). The stability of the cobalt(II)–2,2′,2″-terpyridine complex is increased by using a nitrobenzene extraction (*404*). The green carbonatocobalt(III) complex is believed to have the composition of $Co(CO_3)_3^{---}$ (*414*). The absorption spectra of the various complexes formed by cobalt(II) and 1,10-phenanthroline have been obtained (*678*).

The dissociation constants of 8-quinolinol and a number of its methyl derivatives and related compounds have been determined. In addition, the stability constants for some of the complexes which these reagents form have been evaluated (*261*). The effect of substituents on the absorption spectra and the stability of the chelates of 8-quinolinol and its derivatives have been studied (*427–429*). By determining the heats and entropies of formation of several bivalent metal chelates of 2-methyl- and 4-methyl-8-quinolinol the relative strengths of metal-chelate bonds have been ascertained (*282*). The lower bond strength of the 2-methyl-8-quinolinol chelates was attributed to steric hindrance. The chelates formed with disodium ethylenediamine tetraacetate have come in for increased attention. Copper(II) and iron(III) form complexes with ethylenediaminetetraacetic acid which are suitable for colorimetric measurement (*443*, *451*). Either chromium or cobalt can be determined because of the formation of a compound believed to have the composition of $(Co\text{-}EDTA)_7Cr_2O_7$ (*204*). The stability constants of ethylenediamine tetraacetate chelates of nickel, copper, lead, cobalt, cadmium, and zinc and the effect of ionic strength were determined by spectrophotometric methods (*249*). A stable 1 to 1 complex having a yellow color is formed by palladium(II) and ethylenediaminetetraacetic acid (*371*). Chromium(III) forms a complex in which the ratio of chromium(III) to reagent is 1 to 6 (*94*).

Numerous iron complexes have been subjected to structural studies. The complex formed when iron(II) and dimethylglyoxime, H_2Dm, react in solution of pH 6.5 to 8 has the structure corresponding to $Fe(HDm)_2$, in which a hydrogen atom has been displaced from each molecule of dimethylglyoxime (*19*). If the pH is 5.4 to 6.5 and there is an excess of iron(II), a stable complex corresponding to $FeHDm^+$ is formed. In basic solution, a complex corresponding to the formula of FeDm is formed in the presence of excess dimethylglyoxime. The instability constant for the bis(2,2′,2″-terpyridine)–iron(II) complex has been calculated as being 1×10^{-18} (*74*). Picolinic acid (HR) forms an orange complex of the FeR_3^- type with iron(II). Quinaldic acid forms an analogous compound (*577*). Iron(II) complexes of several α-diimines were prepared and their absorption spectra obtained (*332*). The nature of the complexes formed by acetylacetone (R) and iron(III) have been shown to be a function of the pH of the solution. At low pH the complex is FeR^{++}; at intermediate pH, FeR_2^+; and at higher pH, FeR_3 (*25*). The absorbance maximum shifts from 485 mμ for the FeR^{++} complex to 440 mμ for the FeR_3 complex.

Formulas for iron(III) complexes formed by a number of phenols have been determined by Job's method (*271*). A method of calculating equilibrium constants for complexes such as the ferrisulfosalicylic acid complexes by means of Beer's law and isosbestic points has been outlined (*3*, *643*). The formation constants for the iron(III) sulfate complexes have been determined (*658*). It was established that the predominant specie was $FeCl_4^-$ when iron(III) perchlorate solutions were treated with hydrochloric acid. Molar absorptivities for the $FeCl_4^-$, $FeCl_3$, $FeCl_2^+$, and Fe^{+++} species were computed (*174*). Iron(III) and benzoylacetophenone forms a 1 to 1 red-violet complex having an absorptivity value of 1060 ± 5 at 505 mμ and a dissociation constant of $5.8 \pm 1.6 \times 10^{-12}$ (*351*). The iron thioglycolate complexes have been investigated. In basic, air-free solutions (pH of 9 to 11) a red complex of the $Fe(RS)_2^{--}$ type and a yellow complex of the $FeOHRS^-$ type are formed, where H_2RS represents the reagent (*347*). In acidic solution a precipitate (FeRS) forms. In basic, air-saturated solutions, the complex is presumably $FeOH(RS)_2^{--}$ indicating that an iron(III) complex is formed. Iron(III) catalyzes the air oxidation of H_2RS to $(HRS)_2$. In aqueous solution the formula of the iron(III)–phenol complex is $Fe(OC_6H_5)_3$ (*412*). The dissociation constants of iron(III) complexes of malonate, citrate, and tartrate were determined using iron-56 as a tracer. The tartrate complex is the most stable (*612*). The constitution and complexes formed between iron(III) and organic complexes such as salicylic, hydroxybenzoic, citric, lactic, and tricarboxylic acids have been ascertained and the corresponding equilibrium constants determined (*49*).

A study of the colored complex resulting when vanadate solutions are treated with hydrogen peroxide showed that the vanadium–hydrogen peroxide ratio is 1 to 1 in acidic solution and 1 to 2 in basic solution. The color is less intense in basic solution (*23*). The constitution of the titanium–hydrogen peroxide complex was found to be independent of the acidity and the anion present. The titanium–hydrogen peroxide ratio was found to be 1 to 1, but an excess of hydrogen peroxide is necessary to diminish the dissociation of the complex, which is positively charged and has a dissociation constant of 5.4×10^{-5} (*80*). The formation of a complex of the TiR_6 type has been reported for the reaction of titanium(IV) with gallic acid (*646*). In very dilute solutions of titanium(IV) and thiocyanate a colorless complex having the formula of $TiOH(SCN)^{++}$ is formed. It was observed that the complex exhibited maximum absorptivity at 278 mμ and was extractable with isoamyl alcohol (*127*). The nature of the colored thiocyanate complexes of iron, cobalt, molybdenum, and tungsten has been reviewed by Babko (*18*). The structure of the cobalt(II) thiocyanate complex has been studied (*693*).

The chelates of zirconium and hafnium having the composition MK_4, where K is the particular diketone used, were prepared and their absorption spectra studied. The diketones used were acetylacetone, trifluoroacetone, and 2-furoylacetone. A bathochromic shift was observed for the fluorinated derivatives and a slight bathochromic shift for the zirconium chelates in respect to the hafnium chelates (*342*). In the complexes of uranyl ions with β-diketones covalent bonds exist between the central atom of uranium and the ligands. The uranium(VI) exhibits a coordination number larger than 6 (*544*). A study of the distribution of praseodymium between the aqueous phase and the organic phase of thenoyltrifluoroacetone showed the tris chelate to be the limiting structure (*304*). The nature of the complexes formed by nickel and sodium 2-nitroso-1-naphthol-4-sulfonate has been studied (*628*). In solutions of pH 4 and less a 1 to 1 complex is formed, while in solutions having a pH of 6 and higher the nickel to reagent ratio is 1 to 3 (*627*). The soluble nickel compound formed with dimethylglyoxime in the presence of a number of different oxidizing agents has a ratio of reagent to nickel of 3 to 1 at maximum absorbance (*422*). It was suggested that the agent oxidized nickel(II) to nickel(III), which formed a compound having the formula of $Ni(Dm)_3$.

The compositions of the dithizonates of nickel, cobalt, tin(II), bismuth, copper(II), silver, and mercury(II) have been determined by the spectrophotometric method. Dissociation constants for these dithizonates have also been determined (*501*). As the result of an ion exchange and absorptiometric study it was found that at moderate thiocyanate concentrations only mononuclear complexes of nickel(II)—namely, $NiSCN^+$, $Ni(SCN)_2$, and $Ni(SCN)_3^-$—are formed (*169*). The dissociation constant of the metal complexes formed with nitrilotricarboxylic acids has been determined for the chelates of nickel(II), copper(II), cobalt(II), cadmium, magnesium, and zinc using the Bjerrum method (*95*).

A spectrophotometric study of the complex formed by chromium(III) and sulfosalicylic acid, which has an absorbance maximum at 550 mμ, has been made (*352*). The equilibrium constants for the reactions of chromium(VI) in acidic solution have been determined (*629*). A complex of cerium(IV) and chromium(VI) having the formula of $CeCrO_4^{++}$ has been investigated and found to be more stable than the cerium(IV) sulfate complex (*630*). The ratio of chromium(III) to oxalate was found to be 1 to 3, and its absorbance maximum was at approximately 600 mμ (*524*).

A spectrophotometric study of the chloro complexes of palladium(II) has shown that at least six complexes exist, ranging from $PdCl^+$ to $PdCl_6^{-4}$ (*608*). Both calcium and magnesium form complexes with Eriochrome Black T with cation to dye ratios of 1 to 1, 1 to 2, and 1 to 3 (*688*). An investigation of the absorption spectrum of phosphorus pentabromide in several solvents has shown that the pentabromide exhibits an absorptivity which is not limited to that of free bromine resulting from dissociation (*507*). The absorption spectra of pyrylium salts have been obtained (*436*). The light absorption of iodine in liquid hydrogen fluoride and in diethyl ether solutions containing hydrogen fluoride has been investigated (*303*). The effect of solvent on the stability of the triiodide ion has been studied, and possible reasons for the decrease in stability with increasing water concentration have been given (*302*). The absorption spectra of iodide and several copper(II) salts have been obtained using liquid ammonia as the solvent (*283*). The absorption spectra of methemoglobin and fluoride, thiocyanate, cyanide, and nitrite salts of methemoglobin have been determined for the 230- to 1100-mμ region (*231*). The relationship between absorption spectra and the chemical structures of conjugated polyenes, *p*-polyphenyls, and unsaturated aldehydes, ketones, and carboxylic acids has been determined (*237*). The visible absorption spectra of chlorophyll-α and -β in pyridine have been studied (*655*). Using Wessenberg photographs the structure of the 9(18)-heteropoly anion in potassium 9(18)-tungstophosphate was found to have the formula of $(P_2W_{18}O_{62})^{-6}$. The tungsten atoms are surrounded by octahedra of oxygen atoms, while the phosphorus atoms are surrounded by tetrahedra of oxygen atoms (*125*). The decomposition of nitric oxide with decreasing pressure has been followed by spectrophotometry (*205*).

Reagents. The uses and properties of 1,10-phenanthroline, 2,2′-bipyridine, 2,2′,2″-terpyridine, and their derivatives as colorimetric reagents were discussed by G. F. Smith in his Fisher Award Address (*591*).

Dipicrylamine gives an insoluble salt with potassium, which can be dissolved in acetone. Conformity to Beer's law is observed for the system using the procedure outlined (*186*). The nature of interfering ions in using the dipicrylamine as reagent for potassium has been studied (*149*). A red dye, Zolon, is reported to give intense blue compounds with silver and copper(I), which indicates some interesting analytical possibilities (*184*). Silver gives a precipitate with 5-(*p*-diethylaminobenzylidene)-rhodanine, which when dissolved in a potassium cyanide solution gives a yellow color suitable for measurement (*529*). The use of rubeanic acid as a colorimetric reagent for cobalt, nickel, palladium, and silver has been investigated (*673*).

It has been reported that 1-*o*-carboxyphenylazo-2-naphthol-3,6-disulfonic acid reacts only with calcium to give a yellow color suitable for the detection and colorimetric determination of calcium (*10*). The use of chloranilic, or bromanilic, acid as a colorimetric reagent for determining traces of calcium has been investigated (*335*). Chloranilic acid has also been used as a reagent for determining strontium; the decrease in the color of the chloranilic acid solution as strontium chloranilate precipitates is proportional to the strontium concentration (*358*). Trace amounts of silver, copper(I), mercury, gold, and palladium give colors with thio-Michler's ketone, *p,p*-bis(dimethylamino)-thiobenzophenone (*185*). Dithizone has been used in the determination of silver, mercury, and copper (*168*).

Urone (*637*) studied the stability of *sym*-diphenylcarbazide in various solvents and recommends ethyl acetate and acetone. Absorption spectra have been used to determine the suitability of 5-nitroso-oxine as an analytical reagent (*260*).

1-(2-Pyridylazo)-2-naphthol has been suggested as a colorimetric reagent for metals, inasmuch as it forms colored chelates with at least 20 metal ions (*100*). A sensitive color reaction has been observed when zinc reacts with *o*-toluidine or *p*-toluidine (*255*).

Triphenylmethylarsonium thiocyanate has been used for the determination of small amounts of cobalt or copper (*142, 143*). Traces of copper in the presence of many metals have been determined using 2-isatoxime methyl ether and extracting the colored complex with chloroform (*136*).

2-Nitropyridine gives a rather specific color with cadmium and it is suggested that dinitropyridine should be a better reagent (*193*). Thiuram, tetramethylthiuram disulfide, is a new colorimetric reagent for copper. The precipitate formed with the reagent is extracted with chloroform and gives a yellow-brown color (*219*). Tetraethylthiuram disulfide is also a specific reagent for copper (*400, 401*). 2-Methyl-8-quinolinol was found to be superior to 8-quinolinol for determinaton of copper (*458*). Polythionic acids form pyridine complexes with copper(II)—e.g., $Cu(Py)_4S_4O_6$—which are blue and should be studied further in respect to spectrophotometric determination of copper, or tetrathionate (*233*). Zircon, 2-carboxy-2-hydroxy-5-sulfoformazylbenzene, gives a blue complex with small amounts of copper and zinc but at different pH values, so that each may be determined in the presence of each other (*542*). Monothiophosphate ions in slightly basic acetate solutions give a blue solution with cobalt(II) (*681*). Biacetylmonoxime and benzilmonoxime have been used in the spectrophotometric determination of cobalt, the absorbance maximum being at 336 and 380 mμ, respectively (*86*). In the case of the α-benzilmonoxime, acetone is used to solubilize the complex. The α-benzilmonoxime complex is extractable with carbon tetrachloride. Less interference with nickel was reported when the benzilmonoxime was used.

α-Furyldioxime is a suitable colorimetric reagent for nickel when a chloroform extraction is incorporated into the procedure (*493*). Dimethylglyoxime is a satisfactory reagent for palladium(II) (*439, 494*). Kirkland and Yoe (*316*) have recommended *p*-nitrosodimethylaniline as a sensitive reagent for platinum. Thiocyanate, with an isoamyl extraction, was used to determine palladium (*516*). The molar absorptivity of dithizone in chloroform is $40.6 \pm 0.5 \times 10^3$ at 606 mμ. This value can be used in calculating excess dithizone, inasmuch as mercury(II) dithizonate does not interfere at this wave length (*340*). The use of 3-mercaptopropionic acid as a reagent for nickel has been examined extensively (*345*). Nitrilotriacetic acid is a reagent for iron, copper, and cobalt (*448, 449, 454*). Hydrobromic acid has been used to determine copper and iron (*444, 445, 450*).

Ethyl-α-isonitrosoacetylacetate reacts with iron(II) in solutions buffered to pH of 7.8 to give a blue complex which is extractable with chloroform (*72*). Iron(III) can be determined following reduction with hydrazine. The color developed when ferric chloride stands with β-hydroxy ketones for a long period is due to β-diketones which are formed in the aging period (*138*). Chrome Yellow O, having the formula 2,3,5-HO-(CH_3)(*p*-HO_3-$SC_6H_4N{:}N)C_6H_2CO_2H$, has been used in determining 0.8 to 5 γ of iron(III) per milliliter (*478*). 5,6-Benzoquinaldinic acid has been suggested for determining 2 to 14 p.p.m. of iron(II), but it does not seem to have any advantages over 1,10-phenanthroline (*377*). 2-Picolinic acid which reacts with iron(II) to give a yellow complex, $Fe(C_6H_4O_2N)_2$, can be used to determine 0.1 to 10 p.p.m. of iron (*374*). Meconic acid forms a complex with iron(III) which obeys Beer's law (*379*). The introduction of the sulfonyl group in salicylic acid results in a more suitable reagent for iron (*575*).

The relative selectivity of 8-quinolinol and 2-methyl-8-quinolinol for Group IIIB metals, when the nitroso group is inserted in the five position, has been investigated (*243*). The diammonium salt of 4,4-bis(3,4-dihydroxyphenylazo)-2,2-stilbenedisulfonic acid has been used for the determination of aluminum (*272*).

For samples containing 1 to 10 p.p.m. of zirconium, Horton (*245*) used 2-(2-hydroxy-3,6-disulfo-1-naphthylazo) benezenearsonic acid (thorin). The preparation and properties of the thorin reagent have been thoroughly investigated (*381*). Oxalohydroxamic acid has been used in the colorimetric estimation of thorium (*134*), and quercetin has been used in the determination of zirconium (*212*) and uranium (*322*). Sulfosalicylic acid has been suggested for the development of a light yellow color with titanium in solutions of pH 4 (*694*).

Salicylhydroxamic acid has also been proposed as a reagent for titanium (*674*). A new method for determining cerium utilizes Sunchromine Pure Blue B, sodium 2,6-dichlorohydroxydimethyl fuchsonedicarboxylate (*409*). Dihydroxy-3,4-phenylazoterphenyl produces a blue-violet precipitate with germanium, which is soluble in nitrobenzene to give a red solution suitable for the determination of 1 to 2 mg. of germanium per liter (*51*). Dibenzoylmethane has been used for determining 1 to 10 p.p.m. of uranium (*687*).

Organic reagents possessing the grouping (OH).C.C(O) or C(OH).C.C(N) form either five- or six-membered heterocyclic inner complexes with tungstate ions (*323*). Thiourea forms yellow complexes with palladium(II), bismuth, tin(IV), and tellurium (*439, 452, 453, 455*).

Several new reagents have been employed in the molybdenum blue reaction. Sulfamic acid is a stabilizer in the molybdenum blue method for phosphorus when a sulfite-chlorostannous reduction is employed (*24*). Diphenylcarbazone reduces molybdogermanic acid to molybdenum blue (*130*). The use of the heteropoly acids in colorimetric analysis has been reviewed by Jean (*276*).

In studying various methods of preparing Nessler's reagent it was observed that potassium iodide decreases the sensitivity of the method (*80*).

Cyanogen halides can split pyridine rings to form glutaconaldehyde derivatives which will couple with 1-phenyl-3-methyl-5-pyrazolone to give an intense magenta to blue color (*150*). Thus, cyanides can be determined after conversion to the cyanogenhalide.

A reagent of potassium iodide and cesium chloride gives a compound having the formula of Cs_2TeI_6 with tellurium. Selenium must be absent (*186*). Tetrabromochrysazin, which forms a 1 to 1 complex with boron, is suitable for the colorimetric determination of 1 to 10 γ of boron per 10 ml. (*685*). A number of phenols have been studied as reagents for nitrates (*65*). The effect of acidity, reagent-nitrate ratio, and time of reaction were found to be pronounced.

Eighteen iron(III) complexes were investigated as reagents for the determination of fluoride by measuring the extent of bleaching. Resorcylaldoxime and 5-phenylsalicylic acid gave the most reproducible results for 0 to 6 p.p.m. of fluoride (*438*).

Among the representative reagents used in the determination of organic constituents, isonicotinic acid has been used for determining 4,3-ketosteroids such as progesterone and testosterone propionate (*636*). Blue tetrazolium has been used for steroids (*399*). A new test for unsaturation has been suggested which employs a halogen–cobalt(II)–acetic acid solution which gives a blue color as chloroacetoxylation takes place (*118*). The color reactions of bromonitroindandione with carbazole, indole, and pyrrole have been studied (*639*). The use of anthrone as a reagent for ribose, deoxyribose, and nucleic acids has been investigated (*179*).

Preliminary Treatments. In preparation for colorimetric determinations of a desired constituent considerable preparative chemistry may have to be used. A few examples of two kinds of such preliminary treatment are included.

PROTECTIVE REACTIONS. In the determination of iron with thiocyanate the interference caused by phosphate ions can be eliminated by the addition of bismuth(III) ions (*512*). In the direct determination of aluminum with aluminon the interference by iron(III) can be eliminated by addition of hydroxylamine and 1,10-phenanthroline in acetic acid solution of pH 4.7 (*248*). The interference of iron(III) in the determination of titanium with chromotropic acid can be eliminated by reducing to iron(II) with ascorbic acid (*320*). In determining lead in urine, using dithizone, fresh solutions of sodium cyanide inhibit the extraction of the lead with dithizone. Aging of the cyanide solution for at least one week is recommended. In collecting air samples the substitution of hydrochloric acid for nitric acid in the solution used for collecting the sample minimized the interference of tin in the determination of lead unless the bismuth was assimilated in a prolonged period (*141*). The excess alizarin used in the determination of aluminum can be removed by extracting with ether (*22*).

Elimination of the interference of iron, copper, nickel, cobalt, chromium, and platinum in the determination of palladium with 2-nitroso-1-naphthol can be accomplished by using ethylenediaminetetraacetic acid at pH 2.5 before extracting the palladium complex with toluene (*99*). A solution containing ethylenediaminetetraacetic acid, cyanide, and ammonia inhibits metals other than bismuth from reacting with sodium diethyldithiocarbamate prior to the extraction of the bismuth complex with carbon tetrachloride (*101*). The use of traces of butyl Cellosolve ameliorates the extraction of the antimony–rhodamine B complex with benzene (*362*).

The state of silicic acid in solution prior to the colorimetric determination of silicon is important. The tendency for polymerization exists for acidic solutions and high concentrations of silica. Molecular dispersion of the silica can be achieved by heating and adding sodium hydroxide (*503*). Depolymerization in acidic solution is possible by addition of sodium fluoride (*502*). The reaction in which cyanide and an aldehyde react instantaneously in a buffered acetate solution to give cyanohydrin has been suggested as a way by which halides can be determined in the presence of cyanides (*613*). Permanganate has been recommended to oxidize phosphorus to the ortho form, with sodium nitrite being used to remove excess permanganate (*110*).

Sodium fluoride has also been suggested as complexer of titanium(IV) in order to prevent the precipitation of titanium hydroxide in the determination of phosphorus in alloys (*110*). The addition of fluoborate eliminates the interference of metals, except copper and gold, in the determination of phosphorus (*362*). Sodium hexametaphosphate eliminates the color due to iron(III) citrate (*46*). In the colorimetric determination of aluminum with 8-quinolinol the correction for the iron quinolinol extracted also by the chloroform can be made by making photometric measurements at 390 and 470 mμ (*9*). Hydrogen peroxide can be used to oxidize arsenic(III) to arsenic(V) prior to determination of arsenic by means of the heteropoly blue method (*537*). The deleterious effect of fluoride in determining the sulfate content of water can be eliminated by adding lanthanum nitrate, while the interfering hydrogen carbonate ions can be removed by a weak acid cation exchange resin (*339*).

Gelatin acts as a protective colloid to prevent the precipitation of toluidine blue in the determination of copper (*63*). The addition of tartaric acid to the reagent and a change in the order of addition eliminates the interference of tantalum in the determination of niobium with thiocyanate (*81*). The substitution of

Table I. Analytical Separations of Inorganic Constituents

Desired Constituent (Metals)	Constituent Separated	Reagent	Method	Ref.
Al	Fe	Thiocyanate, BuOH, and Et_2O	Extrn.	(*22*)
Be(Cu)[a]	Be	Guanadine carbonate	Pptn.	(*202*)
Cu	Cu	Thiocyanate and tributyl phosphate	Extrn.	(*396*)
Cu(Fe)[a]	Cu	1,10-Phenanthroline	Extrn.	(*662*)
Nb(Ti)[a]	Nb	Tannin	Pptn.	(*462*)
Nb(Fe)[a]	Nb	Chloric acid	Hydrolytic pptn.	(*293*)
Pd(Pb)[a]	Pd	Dimethylglyoxime and chloroform	Extrn.	(*167*)
Pd(Pt)[a]	Pb	*p*-Nitrosodimethylaniline	Extrn.	(*316*)
Re(Mo)[a]	Re	Tetraphenylarsonium perrhenate	Extrn.	(*40*)
Sb(III)	Tl(I)	Cupferron and chloroform	Extrn.	(*362*)
Sb(V)	Fe(III)	Cupferron and chloroform	Extrn.	(*362*)
Sn	As, Sb	Hydrochloric acid and hydrazine sulfate	Distn.	(*151*)
Ti (Fe, Ni, Mn, Cu, Co, Cr, Zn, Mg, Al)[a]	Ti		Pptn.	(*5*)
Ti	Ti	Cupferron and chloroform	Extrn.	(*97*)
V(Al)[a]	V	Sodium diethyldithiocarbamate and chloroform	Extrn.	(*147*)
Zr	Zr	*p*-Dimethylaminoazobenzenearsonic acid	Pptn.	(*212*)
As	As	Chloride	Voltn.	(*475*, *537*)
	P	Molybdate and mixt. solv.	Extrn.	(*314*)
	As	Diethylcarbamate and chloroform	Extrn.	(*362*)
	Ge	Chloride	Voltn.	(*362*)
B(Fe)[a]	Fe	Mercury cathode	Elec.	(*578*)
	B	Methanol	Distn.	(*578*)
F	F	Acid	Distn.	(*170*, *211*)
ClO_2	Fe	Sodium pyrophosphate	Pptn.	(*234*)
B (metals)[a]	B	Methanol	Distn.	(*683*)
NO_3^- (metals)[a]		Amberlite IR-4B	Ion Exch.	(*285*)
P	Fe	Mercury-cathode	Elec.	(*181*)
	As	Chloride	Voltn.	(*223*)
	Si	Acid	Pptn.	(*183*, *362*)
	Si	Fluoride	Voltn.	(*110*)
	P	Butanol and chloroform	Extrn.	(*650*)
	U	Dowex 50	Ion Exch.	(*113*)
	Ge, Se, Sb, As	Halides	Voltn.	(*223*, *362*)
Se(As)[a]	Se	Bromide	Distn.	(*263*)
Te	Te	Diethyldithiocarbamate and carbon tetrachloride	Extrn.	(*65*)

[a] Parentheses indicate principal diverse element(s).

pyridine for ammonia gives a more stable color using dimethylglyoxime and iron(II) and facilitates extraction with chloroform (*470*).

SEPARATIONS. The ever-present problem of trying to partition the desired constituent and interfering substances has continued to tax the ingenuity of analytical chemists. In addition to a few representative separations tabulated in Table I, several interesting examples of analytical separations applicable to colorimetric analysis are included.

The optimum pH ranges for the complete extraction of nickel dimethylglyoxime by chloroform has been found to be 4.7 to 10 for pure nickel solutions, 4 to 12 for tartrate solutions, and 7.2 to 12 for citrate solutions (*106*). Traces of silver, mercury, and copper can be separated with dithizone (*168*). The complexant, ethylenediaminetetraacetic acid, prevents the interference of iron, cobalt, and nickel in subsequent extractions with diethyldithiocarbamate in chloroform (*108*). The theory underlying the extraction of associated and nonassociated complexes has been discussed (*671*). The interference of iron(III) and cobalt(II) in the determination of nickel with dimethylglyoxime can be eliminated by the addition of *N*,*N*-bis(2-hydroxyethyl)-glycine which forms chelates with these ions (*85*).

Manganese and chromium were separated from brine solutions by precipitating with ferric hydroxide. After reduction, the iron(II) and manganese(II) were separated from chromium as the chromium(III) hydroxide was coprecipitated with aluminum hydroxide (*326*). Molybdenum was separated from various interfering ions by precipitating with α-benzoinoxime prior to determination with Tiron reagent (*664*). Aluminum can be separated from chromium or iron by precipitating as aluminum hydroxide at pH 5.8 to 6.8 in the presence of iron, and at pH 6.2 to 7 in the presence of chromium. The precipitation of iron, or chromium, as hydroxide is inhibited by complexing with 2-picolinic acid (*375*).

A method of separating monocarboxylic acids differing by two carbon atoms in the range C_{13} to C_{16} and dicarboxylic acids in the range C_2 to C_{22} on silicic acid columns using methyl Cellosolve-water as the internal phase and Skellysolve B, *n*-butyl ether, or mixtures of these solvents as the external phase, has been described (*692*).

PHYSICS

The physical aspects underlying the measurement of light absorption are considered under the following headings: laws of absorption, principles of measurement, and instrumentation.

Laws of Absorption. Liebhafsky and Pfeiffer (*353*) have derived Beer's law using the number of "absorbing centers" rather than concentration as being indicative of the amount of absorbing material. Thus, the expression, $A = \log I_0/I = BN/a$, where N is the number of absorbing identical centers acting independent of each other, a is the cross-sectional area of sample containing N centers, and B is the proportionality constant, is applicable to homogeneous as well as nonhomogeneous systems. This viewpoint, actually the original concept, extends the applicability of Beer's law. Goldstein and Day (*197*) have given a kinetic interpretation to the Bouguer-Beer law according to which the number of photons absorbed per unit time is proportional to the number of collisions between molecules and photons, a first-order process. Such an interpretation of the absorption law somewhat simplifies the explanation of the variation in transmittance with wave length and the width of absorption bands. Suggestions for experiments on the laws of light absorption have been given (*62*).

When the number of molecules encountered by each light ray is not constant, errors are more likely to result from rigid application of the absorption law (*534*). Ross (*539*) has treated rather rigorously the application of the absorption law to samples of nonuniform concentration. Amiot and Blondeau (*7*) have discussed the application of Beer's law to colloidal suspensions. A study of the accuracy of photometric analysis showed that large, variable errors result in the presence of colloids (*157*). Nonconformity of the absorption law to suspensions of powders in a liquid has been attributed to the high reflectivity of the powder (*534*). Deviation from Beer's law by dyes has been discussed (*540*).

Goldenberg (*195*) has proposed a method of rectification for nonlinear Beer's law plots by use of the formula, $C = \frac{A}{K-A} \times \frac{1}{m}$, where A is the absorbance and K and m are constants for a specific system and instrument.

Causes for anomalies in the Beer-Bouguer law have been discussed (*196*, *641*).

Principles of Measurement. Reilley and Crawford (*523*) discuss the fundamentals of precision spectrophotometry and point out that with a constant source, the manner of using the slit width, sensitivity, and dark current controls affect the precision of measuring concentration. They propose two additional methods of precise measurement in addition to the classical "0–100" method and the "transmittance ratio" method. In their "trace analysis" method the spectrophotometer is adjusted to "100%" T when the beam passes through the pure solvent and to "0%" T when the beam passes through a solution slightly more concentrated than the sample solution to be measured. The second proposed precision method, termed the "general" method, involves the use of two reference solutions in adjusting the "0%" T settings. A rapid, graphical absorbance-ratio method has been described for a two-component spectrophotometric analysis in which the ratio of the observed absorbances at two selected wave lengths is used (*239*). An "isoabsorptive" point is selected as one wave length whenever possible. A short spectrophotometric method for determining one component of a mixture has been described (*4*).

A new differential method for precision colorimetric analysis, suggested by Ringbom and Osterhold (*530*), always utilizes the maximum sensitivity of the photocell and requires only medium absorbance standards. The matching and positioning of cuvettes are not critical, and conformity to Beer's law is not necessary. The technique of this differential method is more complicated than the differential method recommended by Hiskey and his coworkers. In this new method the instrument is adjusted to "0%" T using a reference standard solution of medium absorbance in the cuvette. Water is then added in definite increments to the standard solution, with the increase in transmittance being noted. Thus, the relationship between transmittance and concentration, termed α value, is obtained. Then, after either adjusting the instrument to "0%" T if the sample solution is less concentrated than the diluted standard solution, or to "100%" T if the sample solution is more concentrated than the diluted standard solution, a known volume of the sample solution is added to the diluted standard solution in the cuvette and the resulting change in transmittance recorded. If the change in deflection due to addition of sample solution is termed β, if K is an experimentally determined constant, and c_2 is the diluted standard solution, it is possible to calculate the concentration of sample solution from the following expression, $c = c_2\left(1 + K\frac{B}{\alpha}\right)$. Chulanovskiĭ (*105*) has also described a method for analyzing mixtures when the absorption bands partly overlap.

The use of nomograms for the spectrophotometric analysis of a two-component system where an isosbestic wave length is not available, or is available, is outlined by Ohmata (*468, 469*).

The relative advantages of visual and photoelectric color measurements have been discussed (*217*). Milazzo (*403*) has made a critical comparison of photographic and photoelectric apparatus and methods. The nature of errors and the use of statistics in the critical evaluation of spectrophotometers have been outlined (*562*). The general principles involved in increasing the precision of spectrophotometric measurements have been presented (*594*). The maximum accuracy in an absorbance measurement does not always exist at 0.434, because the point of least error depends on two parameters. One parameter is affected by variations in the source, sensitivity of the detector, and errors in null point adjustment while the other parameter depends on visual error in reading the instrument (*696*). The effect of slit width upon the intensity and resolution of the spectrum and the results of a calibration study on different types of slits have been discussed (*418*).

The results of a collaborative test in which 72 different spectrophotometers were used to check accuracy and precision with potassium dichromate solutions as a reference standard have been described (*209, 224, 357, 417*). Suggestions and precautions in obtaining reliable values and in conducting such studies have been cited. For the wave lengths 235, 257, 313, and 350 mμ, absorptivity values (1%, 1 cm.) of 125.2, 145.6, 48.9, and 107.0 have been given as being most acceptable for the potassium dichromate system. A comparative survey of spectrophotometers in the 210- to 760-mμ region has been made using a potassium chromate solution and a sample of Trapex glass. Relatively larger deviations were obtained at wave lengths below 235 mμ, probably because of use of wider slit widths. The wider slit widths are necessary because of the absorptivity of the solvent, the decrease in the sensitivity of the phototube, and the larger amount of scattered light at the shorter wave length (*75*). A collaborative study of Cary spectrophotometers using glass filters showed satisfactory agreement among the photometric scales of the instruments used (*640*).

In making spectrophotometric measurements on steep absorption curves with the Beckman spectrophotometer it is suggested that the wave length setting be approached from the same direction to avoid serious transmittance errors (*355*). A review on photoelectric spectrophotometric measurements includes a discussion of errors due to stray light and fluorescence (*308*). The effect of temperature on the absorption spectra of potassium chromate has been investigated (*635*).

Ovenston (*479*) studied the effect of fluorescence, with special attention to the distance between photocell and origin of fluorescence. In general, the fluorescence error increased with decrease in distance between solution and photocell, but for spectrophotometers such as the Beckman DU, Unicam SP500, and Uvispek, accurate absorption measurements are possible in the optimum working range of 0.2 to 0.8 absorbance provided absorption cells are placed in the proper position.

Mehler (*391*) has indicated that the potential errors in the measurement of high absorbance systems can be attributed to stray light when a multiplier phototube detector is used. Thus, when a high fraction of the total radiant energy reaching the tube is due to stray light, changes in the selected monochromatic radiant energy will have slight effect on the output. The use of special light sources, filters, or additional monochromators reduces stray light. The stray light in spectrophotometers caused by a 60° prism amounts to about 0.1% of the total energy of the spectrum (*140*).

The Spekker Absorptiometer, Models 560 and H 760, gave a linear response with a mercury light source but not with a tungsten bulb, because the latter fluctuated in intensity (*563*).

Unique methods of checking the wave-length and absorbance scales of spectrophotometers have been evaluated (*232*). The wave-length scale is calibrated using the absorbance wave of an interference pack. The absorbance scale is checked by using several wire screens which can be oriented about the optical axis. Hansen (*222*) has described a process by which an auxiliary source is superimposed on the light beam in order to test the linear relation between intensity and indicated value.

Instrumentation. Progress has been made in the development of attachments for commerically available instruments, several new spectrophotometers have appeared, and a number of special application instruments have been described.

FILTER PHOTOMETERS. The preparation of photofilters with predetermined transmittance curves has been given (*71*). A photoelectric photometer employing a sensitive amplifier circuit has been described (*295*). Another photometer has a 7.7-cm. absorption cell and requires only 2 ml. of solution (*241*). A filter photometer has been modified with a servo-amplifier so designed that the difference in the voltage of the two photocells is inverted to 60-cycle alternating current and amplified to operate a slide wire servo-motor. A per cent transmittance scale is attached to the motor (*595*). A photoelectric photometer suitable for biochemical determinations uses a selenium and a silver sulfide cell to cover the 320- to 750-mμ and 700- to 1200-mμ regions, respectively. Variable sensitivity is achieved by shunting the galvanometer (*645*). An inexpensive interference type of spectrophotometer has a spectral band width of about 10 mμ (*545*). The commercially available Kruger Model 61 photometer employs a wedge interference filter, gives a spectral band width of about 15 mμ at 550 mμ, and has a wave-length range of 420 to 680 mμ (*329*). Oster (*476*) designed a universal, high-sensitivity photometer for measuring light scattering, luminescence, transmittance, and reflectance. An excellent summary of essential information on the maintenance, standardization, and use of photoelectric photometers is given by O'Hagen (*467*).

SPECTROPHOTOMETERS. A number of new American instruments warrant special mention. The Beckman ratio recording spectrophotometer, Model DK-2, records spectra in the 200- to 3000-mμ region using a flat-chart recorder while the Model DK-1 utilizes a strip-chart recorder (*37*). The use of the Beckman DU monochromator with various automatic recording devices has been reviewed by DeVos (*132*).

The Warren Spectracord uses a beam splitter and chopper at the exit slit of a Beckman monochromator in order to obtain modulated double-beam performance. In the ratio recording system a sufficient part of the reference signal is added to the sample channel in order to increase its signal to equal that of the reference channel. The ability to adjust electrically the 0 and 100% transmittance levels by means of a potentiometer control is an outstanding feature of the unique circuitry employed. The speed, reproducibility, and resolution obtainable with this instrument are excellent (*541*). With the Warren Spectracord the ultraviolet (210 to 370 mμ) or the visible (350 to 750 mμ) regions can be recorded, depending on the source used. An attachment enables measurements to be made in the near infrared region to 2.75 microns.

The new General Electric spectrophotometer scans the 380- to 700-mμ region, in 54 seconds to $2^1/_3$ minutes. Increased sensitivity over the previous model has been achieved by a new phototube and the use of a Plexiglas rod extending into the integrating sphere. The rod reflects the light from the walls of the integrating sphere directly to the phototube. The new electronic system eliminates the use of thyratron tubes (*305, 513*).

There are new Cary spectrophotometers, Models 13 and 14. Model 14 has a double monochromator consisting of a 30° prism in series with a 600 line per mm. echlette grating. A single IP28 multiplier phototube with an attenuated beam gives a photometer which alternately measures the radiant energy transmitted by sample and reference solutions. With the lead sulfide cell for the near-infrared region the Cary Model 14 will record from 190

Table II. Photometric Methods for Metals

Constituent	Materia	Method or Reagent	References
Ag	...	Nitroso-R salt	(*548*)
	...	Dithizone	(*146, 168*)
	Ores	5-*p*-Diethylaminobenzylidene rhodanine	(*529*)
Al	...	Thiosalicylic acid	(*242*)
	...	Aurintricarboxylic acid	(*242*)
	...	Morin	(*615*)
	Iron and steel	8-Quinolinol	(*9, 107, 291, 533*)
	Iron and steel	2-Carboxy-2-hydroxy-5-sulfoformazylbenzene	(*542*)
	Steel	Stilbazo	(*272*)
	Steel	Aluminon	(*424*)
	Titanium alloys	Aluminon	(*111*)
	Zinc	Aluminon	(*490*)
	Phosphate materials	Ferron	(*128*)
	Clays	Eriochrome cyanine	(*194*)
	Building materials	Aluminon	(*248*)
	Thorium	8-Quinolinol	(*382*)
Ba	...	Molybdate-thiocyanate	(*463*)
Be	...	Chrome Azurol-S	(*669*)
	Copper alloy	Aluminon	(*202*)
Bi	...	Thiourea	(*447*)
	...	Hydrochloric acid	(*398*)
	Lead alloy	Ethyldithiocarbamate	(*101*)
	Lead-tin alloys	Thiourea	(*11*)
Ca	...	Ammonium purpurate	(*1, 665*)
	...	Iron(II) salicylate	(*83, 383*)
	...	Chloranilic acid	(*335*)
	...	Disodium 3-(4-chloro-1-hydroxy-2-phenylazo)-4,5-dihydroxy-2,7-disulfonate	(*679*)
	Serum	Alizarin	(*430*)
	Serum	Chloranilic acid	(*622*)
Ce	...	Lead dioxide oxidation	(*198*)
	...	Sodium 2,6-dichlorohydroxy-dimethylfuchsonedicarboxylate	(*409*)
Co	...	Thiocyanate	(*258*)
	...	Ethylenediaminetetraacetic acid	(*204, 511*)
	...	2-Nitroso-1-naphthol-4-sulfonic acid	(*667*)
	...	Oxalate	(*524*)
	...	2-Methyl-2-thiopseudourea sulfate	(*582*)
	...	Nitroso-R salt	(*162, 486, 579*)
	...	2,2′,2″-Terpyridine	(*404*)
	Biological material	Nitroso-R salt	(*548*)
	Steel	Thiocyanate, isopropylketone	(*315*)
	Ores	Thiosulfate-phosphate	(*172*)
	Alloys	Nitrilotriacetic acid	(*448*)
	Nickel	Tetraphenylarsine chloridethiocyanate	(*277*)
	Steel	Thiocyanate–isoamyl alcohol	(*236*)
	Alloys	Triphenylmethylarsonium thiocyanate	(*142*)
	Gastric juice	Thiocyanate	(*176*)
	Fodder	2-Nitroso-1-naphthol	(*33*)
	Fertilizer	Nitroso-R salt	(*46*)
	Soils	Nitroso-R salt	(*82*)
	Steel	Isonitrosomalonylguanidine	(*275*)
Cr	...	Diphenylcarbazone	(*70*)
	...	Diphenylcarbazide	(*465*)
	...	Ethylenediaminetetraacetic acid	(*204*)
	Brine	Diphenylcarbazide	(*326*)
	Platinum catalysts	Dichromate	(*574*)
	Effluents	Diphenylcarbazide	(*566*)
	Steel	Chromate	(*354*)
	Steel	Diphenylcarbazide	(*208*)
	Biological media	1,5-Diphenylcarbazide	(*213*)
	Bronze, steel	Peroxide	(*290*)
Cu	...	Dithizone	(*168, 541*)
	...	Thiobenzamide	(*171*)
	...	Diethyldithiocarbamate	(*273, 278, 576*)
	...	Diazobenzenesulfonic acid and dimethyl-8-quinolinol	(*44*)
	...	Ethylenediamine tetraacetate	(*443, 451*)
	...	Hydrobromic acid	(*444, 450*)
	...	Neocuproine	(*173*)
	...	2-Methyl-8-quinolinol	(*458*)
	...	Nitrilotriacetic acid	(*449*)
	Steel	*o*-Tolidine-thiocyanate	(*63*)
	Steel	Neocuproine	(*173*)
	Alloys	Triphenylarsonium thiocyanate	(*143*)
	Metals	Diethyldithiocarbamate	(*108*)
	Bearing metal	Diethyldithiocarbamate	(*244*)
	Metals	2,2′-Biquinoline	(*215*)
	Semiconductors	Neocuproine	(*362*)
	Copper alloy	Dimethylglyoxime	(*292*)
	White metal	Ammonia	(*660*)
	Lead alloy	Ammonia	(*504*)
	Platinum catalysts	Neocuproine	(*574*)
	Steel	Diethyldithiocarbamate	(*208*)
	Tantalum oxides	Diethyldithiocarbamate	(*230*)
	Metal finishing effluent	Mercaptobenzothiazole	(*177*)
	Industrial wastes	Rubeanic acid	(*268*)
	Serum	Biscyclohexanone oxalyldihydrazone	(*495*)
	Water	Cuproine	(*190, 246*)
	Plant materials	Dimethyldithiocarbamate	(*163*)
	Plants	Cuproine	(*246*)
	Biological media	Cuproine	(*246*)
	Cider	Dibenzyldithiocarbamate	(*626*)
Fe	...	Thiocyanate	(*512*)
	...	Formolhydroxamic acid	(*649*)
	...	2,2′-Bipyridine	(*455, 605*)
	...	Hydrobromic acid	(*445*)
	...	Chrome yellow	(*478*)
	...	Nitroso-R salt	(*472*)
	...	Dimethylglyoxime	(*256, 470*)
	...	Nitrilotriacetic acid	(*454*)
	...	Ethylenediaminetetraacetic acid	(*443*)
	...	Meconic acid	(*379*)
	...	Antipyrine-thiocyanate	(*606*)
	...	Sulfosalicylic acid	(*543*)
	...	Cacotheline	(*521*)
	Catalysts	4,7-Diphenyl-1,10-phenanthroline	(*574*)
	Babbitt metal	Sulfosalicylic acid	(*477*)
	Copper alloys	Tartaric acid, sodium periodate	(*441, 442*)
	Steel	1,10-Phenanthroline	(*380*)
	Metal finishing effluent	1,10-Phenanthroline	(*565*)
	Tantalum oxides, tantalum	1,10-Phenanthroline	(*230*)
	Clays	Sulfosalicylic acid	(*194*)
	Plants, soils	Cupferron–isoamyl acetate	(*39*)
	Biological media	1,10-Phenanthroline	(*294*)
	Aluminum alloys	1,10-Phenanthroline	(*267*)
	Aluminum alloys	Isonitrosomalonylguanidine	(*275*)
Ge	...	Phenylfluorone	(*564*)
	...	Querecetin	(*471*)
Hg	...	Dithizone	(*168*)
	...	Ferrocyanide-nitrosobenzene	(*13*)
	Biological media	Dithizone	(*506, 654*)
Ir	...	Cerate	(*389*)
K	...	Dipicrylamine	(*214*)

mμ to 2.6 microns. The scanning speed can be adjusted from 0.5 to 500 A. per second (*8*). Model 13 uses the double quartz monochromator of Model 11 with the photometer and absorption cell optical system of Model 14.

The Bausch & Lomb Spectronic 20 is a new, relatively inexpensive instrument which serves as either a photoelectric photometer or spectrophotometer (*36*). It has a spectral band width of 20 mμ, and with auxiliary infrared-sensitive tube it can be used in the 375- to 950-mμ region, or in the 375- to 650-mμ region with a single phototube. The monochromator consists of a diffraction grating, with a photoemission-type phototube being used with an amplifier instead of a photovoltaic cell–galvanometer type photometer common to most inexpensive photometers. The main disadvantage of this instrument is the inconvenience in changing from reference to sample in scanning the wave length, inasmuch as provision for only one cell has been made in the present model. The transmittance scale is not as large as many chemists would like. The price and utility of this instrument should do much to facilitate training in colorimetry and spectrophotometry in undergraduate courses.

A double-beam microspectrophotometer for samples as small as 0.015 ml. has been developed. A 30,000 line per inch grating serves as the dispersive element while a single multiplier phototube serves alternately as detector of the chopped reference and sample beams. The meter is calibrated in absorbance units. The spectral range is 210 to above 600 mμ with a spectral slit width of 1.5 mμ (*270*).

Beitz (*41*) has described the rapid scanning spectrophotometer

Table II. Photometric Methods for Metals (*Continued*)

Constituent	Material	Method or Reagent	References
Li	...	Ferricyanide	(*161*)
Mg	...	Dimethyl-8-quinolinol	(*44*)
	...	Titan yellow	(*363, 388, 556*)
	...	Thiazole yellow	(*410*)
	...	Disodium-(4-chloro-1-hydroxy-2-phenylazo)-4,5-dihydroxy-2,7-naphthalene disulfonate	(*679*)
	...	4-Aminophenazone	(*368*)
	Soil and plant extracts	2-Thiazole	(*684*)
	Alkali products	Thiazole yellow	(*307*)
	Zinc plate	8-Quinolinol	(*610*)
	Plants	Thiazole yellow	(*550*)
	Nickel	8-Quinolinol-chloroform	(*363*)
	Titanium alloys	Titan yellow	(*97*)
Mn	...	Bromate–Mn(III) sulfate	(*517*)
	...	Dithiocarbamate	(*600*)
	...	Bromate	(*517*)
	Brine	Silver peroxide–permanganate	(*326*)
	Catalysts	Permanganate	(*574*)
	Steel	Persulfate	(*208*)
Mo	...	Mercaptoacetic acid	(*663*)
	...	Thiocyanate	(*116, 162*)
	...	Molybdoferrocyanide	(*525*)
	Steel	Tiron	(*664*)
	Steel	Thiocyanate	(*2*)
	Plants	Thiocyanate	(*281*)
Nb	Steel	8-Quinolinol	(*293*)
	Ore	Thiocyanate	(*385, 386, 406*)
	Rocks	Thiocyanate	(*651*)
	Ores	Peroxide	(*498, 499*)
	Titanium alloys	Thiocyanate	(*462*)
	Steel	Thiocyanate	(*407*)
	Ore	Pyrogallol	(*250*)
	Steel	Hydroquinone	(*259*)
Ni	...	*p*-Phenetidine-ferricyanide	(*254*)
	...	2-Methyl-2-thiopseudourea sulfate	(*582*)
	...	Heptoxime-chloroform	(*191*)
	...	β-Mercaptopropionic acid	(*345*)
	...	Dimethylglyoxime	(*85*)
	Tungsten	Diethyldithiocarbamate	(*532*)
	Catalysts	Dithizone	(*574*)
	Metal finishing effluents	Dimethylglyoxime	(*178*)
	Plants	Dimethylglyoxime	(*162*)
Os	...	Thiourea	(*533*)
Pb	...	Hydrochloric acid	(*398*)
	Urine	Dithizone	(*370*)
	Catalysts	Hydrochloric acid	(*574*)
	Atmosphere	Quinalizarin	(*415*)
	Steel	Turbidimetric-sulfate	(*84*)
Pd	...	Ethylenediaminetetraacetic acid	(*371*)
	...	Dimethylglyoxime	(*494*)
	...	Thiocyanate	(*440, 516*)
	...	Thiourea	(*439*)
	...	Iodide	(*167*)
	...	*p*-Nitrosodimethylaniline	(*686*)
Pt	...	*p*-Nitrosodiumethylaniline	(*316, 686*)
	Catalysts	Hydrochloric acid–tin(II) chloride	(*574*)
Rare earths	...	Thiosalicylic acid, aurintricarboxylic acid	(*242*)
	Fission products	Alizarin red S	(*528*)
Re	Ore	Thiocyanate	(*40*)
Rh	...	Chlorostannous acid	(*389*)
Ru	...	Perruthenate	(*603*)
Sb	...	Methyl violet	(*274*)
	...	Rhodamine B	(*453, 474*)
	Lead	Rhodamine B	(*360*)
	Semiconductors	Rhodamine B	(*362*)
Sn	...	Hematoxylin	(*623*)
	...	Morin	(*488*)
	...	Thiourea	(*452*)
	...	Diethyldithiocarbamate	(*124*)
	...	Dithiol	(*151*)
	...	Hydrobromic acid	(*446*)
	Iron and steel	Cacotheline	(*203*)
Sr	...	Chloranilic acid	(*358*)
Ta	Ore	Pyrogallol	(*250, 386*)
	Titanium alloys	Pyrogallol	(*461*)
	Steel	Pyrogallol	(*259*)
Th	...	Naphthazarin	(*413*)
	...	Quinalizarin	(*519*)
Ti	...	Sulfosalicylic acid	(*694*)
	...	Chromotropic acid	(*594, 580*)
	...	Indirect-Mo blue	(*464*)
	Catalysts	Tiron	(*574*)
	Iron and steel	Sulfosalicylic acid	(*543*)
	Titanium alloys	Peroxide	(*432, 482*)
	Uranium-titanium alloys	Peroxide	(*405*)
	Steel	Peroxide	(*392*)
	Pig iron	Chromotropic acid	(*320*)
	Tantalum-oxides	Peroxide	(*230*)
	Clays	Peroxide	(*194*)
Tl	...	Hydrochloric acid	(*398*)
U	...	Thiocyanate	(*287*)
	...	Reduction	(*88*)
	...	Dibenzoylmethane	(*510, 687*)
	...	8-Quinolinol	(*584*)
	...	Nitroso-R salt	(*423*)
	...	Quercetin	(*322*)
	...	Peroxide	(*88*)
	Concentrates	Peroxide	(*216*)
	Thorium	Thiocyanate	(*510, 583, 687*)
V	...	Catalytic oxidation	(*431*)
	...	Sulfite reduction	(*594*)
	...	Formaldoxime	(*620*)
	...	Pyrocatechol	(*487*)
	...	Peroxide	(*195*)
	Steel	1,10-Phenanthroline	(*538*)
	Steel	Tungstophosphate	(*619*)
	Catalysts	Tungstophosphate	(*574*)
	Water	8-Quinolinol	(*607*)
	Aluminum	Tungstophosphate	(*147*)
W	...	Thiocyanate	(*116, 208,* 460)
	Steel	Thiocyanate	(*392, 581*)
	Steel	Hydroquinone	(*259*)
	Steel	Tungstovanadophosphoric acid	(*281*)
Zn	...	2-Carboxy-2-hydroxy-5-sulfoformazylbenzene	(*542*)
	...	Ferrocyanide and diethylaniline	(*252*)
	...	*p*-Aminophenol	(*253*)
	Effluent	Dithizone	(*421*)
Zr	...	Hematoxylin	(*618*)
	...	2-(2-Hydroxy-3,6-disulfo-1-naphthylazo)-benzene-arsonic acid	(*245*)
	Glass sand	Quercetin	(*212*)

of the American Optical Co. The spectral slit width for absorption work is 6 mμ, with the visible region being scanned in $^1/_{180}$th second 60 times per minute. This instrument is also suitable for reflectance measurements.

Nelson and Jenkinson (*435*) have designed a simple photometer detector for use in direct-recording spectrophotometry. Compensation for changes in energy and detector sensitivity has been achieved by using one of the signals from the single multiplier phototube to adjust the working voltage on the tube so that a constant reference beam signal is maintained.

The conversion of a single-beam infrared spectrophotometer to a ratio-recording ultraviolet and visible spectrophotometer has been accomplished by using a single phototube, a phase-discriminating system to separate the reference and sample beams, and a quartz prism (*313*). A double-beam, concave grating spectrophotometer for the 200- to 1000-mμ region, using the second order, has been built for the measurement of absorption and emission transition probability (*220*). The theory, construction, and use of a logarithmic photometer (*206*) and a monochromator using plane polarized light and the rotatory dispersion of quartz have been described (*251*). A spectrophotometer for the 200- to 1000-mμ region employs a height reducer at the slit for the adjustment of radiant energy without changing the slit width (*500*).

Attachments for the Beckman Model DU spectrophotometer include an attachment which facilitates the examination of liquid films (*367*), a special test tube cell holder (*548*), a photometric titration attachment (*319*), a modified external battery supply (*601*), an automatic device for the measurement of light absorption and fluorescence on paper chromatograms (*78*), and a recording attachment employing a resistor circuit and Brown recorder (*457*).

A linear absorbance potentiometer is interchangeable with the logarithmic absorbance potentiometer of the Beckman DU and Unicam SP 500 spectrophotometers (*416*). A spectral energy recording attachment for the Beckman DU spectrophotometer is also available (*37*).

An attachment permits paper-strip chromatograms to be scanned at predetermined wave lengths or the absorption spectrum of a spot to be recorded with the Cary spectrophotometer (*483*). This spectrophotometer has been used to indicate automatically the equivalence point in the titration of vanadium with coulometrically generated titanium(IV) ion (*378*). The reproducibility of the log density attachment on the Cary spectrophotometer was found to be satisfactory (*164*).

An apparatus by which the absorption spectrum of a sample can be compared with that of a standard sample has been described (*554*).

Special Application Instruments. An analyzer-recorder which measures the darkening of a transparent 16-mm. motion picture film coated with a buffered lead acetate film, as the hydrogen sulfide in air comes in contact with it, has been used to determine up to 100 p.p.m. of hydrogen sulfide (*466*). An instrument employing a microscope and a photovoltaic cell was used to determine sulfuric acid aerosols. The aerosol was impinged on an indicator film containing thymol blue. The change in color was found to be applicable to the determination of 0.01 to 0.2γ of sulfuric acid (*187*). Improved apparatus includes an oximeter for the determination of arterial oxygen saturation (*589*) and assembly for the colorimetric determination of oxygen in nitrogen (*264*). A recording spectrophotometer, suitable for obtaining the absorption spectra of intermediates in biochemical reactions, is operable at a maximum rate of 6 mμ per second (*680*). A special apparatus serves for studying the absorption spectra of flash-initiated reactions for very small time periods (*632*). A modified spectrophotometric apparatus was used in the spectrokinetic study of rapid reactions (*120*). A leucometer measures the pale colors of textiles and powdered samples (*144*). A microspectroscope ocular attached to a microscope was used for examination of pigments at liquid-air temperature (*228*). An apparatus employing a fluorescent tube as source and multiplier phototubes has been utilized for following the change in light absorptivity of chlorine gas with a change in chlorine concentration (*331*). A colorimetric coulometer has been described by Franklin and Roth (*166*); the number of coulombs is proportional to the change in absorbance of a solution. An automatic photometric titrator has been used in titrating calcium and magnesium in carbonate rocks, with disodium ethylenediamine tetraacetate as the titrant (*569*).

A differential absorption meter measures the concentration of very dilute solutions (*393*). A sensitive absorptiometer for measurement of nitrogen dioxide fumes has been described (*587*).

Several instruments specifically designed for use in color specification work and similar applications have been described. An automatic tristimulus photoelectric colorimeter gives the Munsell coordinates (*189*). Thus, hue, value, and chroma are read directly from potentiometer dials. Middleton (*402*) has compared the results obtained with the General Electric spectrophotometer and a Donaldson 45° normal colorimeter using semiglossy specimens. Another tristimulus photoelectric colorimeter measures the chromaticities of pyrotechnic smokes (*459*). The Spectra brightness spot meter manufactured by the Photo Research Corp. utilizes a multiplier phototube in measuring the brightness of low reflectance surfaces (*497*).

Absorption Cells. Several interesting developments concern absorption cells and cuvettes. Included are a thermostated holder for cells in the Beckman DU spectrophotometer (*137*); a matched test tube holder for Beckman DU spectrophotometer (*549*); Teflon inserts to reduce the volume of solutions required in regular absorption cells (*570*); an absorption cell with a funnel top and a drain at the bottom for routine photometric measurements (*58*); a continuous-flow cell for photometric measurements on solutions which fade (*114*); water-jacketed absorption cell holder for use with the Unicam SP 500 (*87*); and an anaerobic cell with which the absorption spectrum of anaerobiosis and the rates of reaction under anaerobic conditions can be measured (*343*).

APPLICATIONS

This section deals with papers on the determination of specific constituents. In many cases, classical methods with modification have been applied to new materials. Several new methods have been developed which may be applicable to a wide variety of samples. In order to document the numerous contributions of this past biennium with a minimum expenditure of space, the reviewers have summarized this information in Tables II, III, and IV.

Attention is also called to Kortum's book, "Kolorimetrie, Photometrie, and Spektrometrie" (*325*) and the paper on spectrophotometry in chemical analysis (*588*).

Permanent Standards. Permanent standards for use in colorimetric methods still get attention. The use of glass standards for the determination of fluoride was facilitated by the proper adjustment of the zirconium and alizarin concentration in the reagent employed (*165*). Solutions of ferrous ammonium sulfate and methyl red serve for the determination of beryllium with aluminon (*202*). Reference standards 2×10^{-5} to 2×10^{-3}-*N* in potassium permanganate have been used in the photometric determination of bilirubin (*341*). Standard solutions of potassium dichromate have been used in the colorimetric determination of carotenoid pigments (*321*). Azobenzene has also been suggested for color standards in the carotene determination (*60*, *567*). In the colorimetric determination of raffinate, standard solutions containing cobalt(II) sulfate, nickel(II) sulfate, ammonium sulfate, and potassium dichromate were used (*408*). Mixed solutions of copper sulfate and bromophenol blue are suitable for the determining of phosphate by the molybdenum blue method (*484*).

Simultaneous and Successive Spectrophotometric Determina-

tions. Polycomponent systems are often amenable to simultaneous or successive spectrophotometric analysis.

The simultaneous determination of aluminum and iron in zinc has been accomplished by measuring the absorbance at 525 and 600 mμ of the colors formed with sodium alizarinsulfonate (*225*). The colored peroxy complexes of niobium and tantalum have characteristic absorbance maxima in the ultraviolet region which are suitable for the simultaneous method (*480*). Benzanthrone and 2,2-dibenzanthronyl can be determined simultaneously when dissolving in sulfuric acid by measuring absorbance at 374 and 550 mμ. The absorbance at these two wave lengths due to dibenzanthrone can be corrected for by making another absorbance measurement at 850 mμ (*657*). The simultaneous colorimetric determination of copper, cobalt, and nickel, using diethyldithiocarbamate, has been described (*103*). The determination of iron(II) and total iron has been performed by using the iron(II) and iron(III) complexes of 1,10-phenanthroline (*229*). Kruse and Mellon (*334*) used a simultaneous spectrophotometric determina-

Table III. Photometric Methods for Nonmetals

Constituent	Material	Method or Reagent	References
As	...	Molybdovanadoarsenate	(*218*)
	...	Molybdoarsenic acid	(*129*)
	Semiconductors	Heteropoly blue	(*362*)
	Biological media	Heteropoly blue	(*148*)
	Silicate rock	Heteropoly blue	(*475*)
	Steel	Heteropoly blue	(*537*)
	...	Silver diethyldithiocarbamate	(*288*)
B	...	Sodium alizarin sulfonate	(*93*)
	...	Tetrabromochrysazin	(*685*)
	...	Curcumin	(*585*)
	...	Quinalizarin	(*384*)
	Semiconductor	Curcumin	(*361*)
	Biological media	Carminic acid	(*593*)
	Soils	1,1′-Dianthrimide	(*199*)
	Steel	Carmine red	(*578*)
	Plants	Tumeric	(*134*, *210*)
	Plants	1,1′-Dianthrimide	(*34*)
	Titanium alloys	1,1′-Dianthrimide	(*112*)
	Silicon	Curcumin	(*361*)
Cl	...	Cadmium iodide–linear starch	(*338*)
	...	Turbidimetric	(*317*)
	...	Mercury(II) thiocyanate	(*638*)
(ClO_3^-)	Ammonium perchlorate	Brucine	(*139*)
(Cl_2)	Atmosphere	Methyl orange	(*328*)
	Water	3,3-Dimethylnaphthidine	(*42*, *238*)
(ClO_2)	Water	Tyrosine	(*240*)
	Water	*o*-Tolidine	
CO	...	NBS indicating gel	(*573*)
	Air	Pd(II) chloride–*p*-nitrosodiethylaniline	(*38*)
COS	...	Methylene blue	(*518*)
(CN^-)	...	Copper(I) thiocyanate–iron(III)	(*638*)
	Wastes	Pyridine-pyrazolone	(*333*)
	Air	Pyridine, 1-phenyl-3-methyl-5-pyrazolone	(*150*)
	Sewage	Benzidine-pyridine	(*104*)
(CNO^-)	...	Nessler's reagent	(*571*)
	...	Pyridine-pyrazolone	(*334*)
$(C_2O_4^{--})$	...	Iron(III) salicylate	(*83*)
F	...	Iron(III) complexes	(*438*)
	...	Thorium–monohydroxyazo dye	(*337*)
	...	Hematoxylin	(*372*)
	...	Iron(III) salicylate	(*131*)
	...	Sodium alizarin monosulfonate	(*61*)
	Water	Zirconium-alizarin	(*170*)
	Water	Aluminum-hematoxylin	(*170*)
	Rocks	Thoron	(*211*)
	Water	Zirconium–Eriochrome Cyanine R	(*390*)
	Water and Soils	Zirconium-alizarin	(*572*)
H^+	...	Universal indicator	(*301*)
H_2O_2	...	Titanium sulfate	(*552*)
	...	Iron(III) thiocyanate	(*489*)
I_2	...	Iron(III) thiocyanate	(*638*)
N	...	Ninhydrin–copper(II) nitrate	(*64*)
	Steel	Nessler's	(*598*)
(NO_2)	Atmosphere	Sulfanilic acid–*N*-(1-naphthyl) ethylenediamine dihydrochloride	(*547*)
(NO_2^-)	...	1-Naphthylamine and phenoldisulfonic acid	(*14*)
	Plants and soils	Sulfanilic acid and 1-naphthylamine	(*434*)
(NO_3^-)	...	Chromotropic acid	(*296*, *614*)
	...	Reduction, aniline and 1-naphthylamine	(*297*, *298*)
	Plants and soils	Sulfanilic acid, 1-naphthylamine	(*434*)
	Plants	2,4-Xylenol	(*285*)
	Water	Chromotropic acid	(*299*)
	Plants	3,4-Xylenol	(*28*)
(NH_4Ac)	...	Sensitized Shiff's reagent	(*55*)
(NH_2OH)	...	8-Quinolinol	(*515*)
(NH_3)	...	Pyridine-pyrazolone	(*334*)
	Water	Phenate-hypochlorite	(*527*, *648*)
O_2	Water	3,3′-Dimethylnaphthidine	(*32*)
	Air	Chromometric	(*419*)
	Water	Leucoindigo carmine	(*17*)
P	...	Molybdophosphoric acid	(*47*, *129*, *437*)
	...	Molybdenum blue-amidol	(*175*)
	...	Molybdovanadophosphoric acid	(*557*)
	Steel	Molybdovanadophosphoric acid	(*26*)
	Organic phosphates	Molybdophosphoric acid	(*47*)
	Water	Molybdenum blue	(*201*, *348*, *514*)
	Silicate rocks	Molybdovanadophosphoric acid	(*16*)
	Iron	Molybdenum blue	(*181*)
	Plant ash	Molybdovanadophosphoric acid	(*188*, *336*, *558*)
	Semiconductor	Molybdenum blue	(*362*)
	Organic compounds	Molybdovanadophosphoric acid	(*366*)
	Biological media	Molybdenum blue	(*411*)
	Copper alloys	Molybdovanadophosphoric acid	(*365*)
	Tooth enamel	Molybdovanadophosphoric acid	(*183*)
	Titanium alloys	Molybdenum blue	(*110*)
	Steel	Molybdenum blue	(*24*)
	Phosphate rock	Molybdovanadophosphoric acid	(*568*)
	Steels	Molybdophosphoric acid	(*650*)
	Alumina	Molybdenum blue	(*145*)
	Silicate rocks	Molybdenum blue	(*156*, *257*)
	Soils	Molybdenum blue	(*690*)
$(P_2O_7^{----})$	...	Molybdenum blue	(*158*)
$(H_2PO_2^-)$	...	Molybdenum blue	(*555*)
PO_3^-	...	Iron(III) thiocyanate	(*624*)
S	...	Sodium nitroprusside	(*546*)
	...	Silver thiocyanate	(*638*)
	...	Mercury(II) thiocyanate and iron(III)	(*638*)
	...	Lauth purple	(*300*)
	...	Barium chromate–diphenylcarbazide	(*266*)
	Biological media	Barium chloride, potassium chromate	(*369*)
	Water	Thorium borate and amaranth	(*339*)
$(S_2O_3^{--})$	...	Copper(I) thiocyanate and iron(III)	(*638*)
	...	Iron(III) alum	(*638*)
	...	Mercury(II) thiocyanate and iron(III)	(*638*)
$(S_4O_6^{--})$	...	Copper(II)-pyridine	(*233*)
SCN^-	Wastes	Copper-pyridine	(*333*)
Se	...	Iodide	(*186*)
	...	Diaminobenzidine	(*247*)
	Magnesium alloys	Sulfuric acid	(*659*)
	Crude copper	Pyrrole-phosphoric acid	(*611*, *612*)
	Sea water	Phenol and thiourea	(*263*)
Si	...	Molybdosilicic acid and Mo-blue	(*35*)
	...	Molybdosilicic acid	(*129*)
	Steel	Molybdenum blue	(*192*, *668*)
	Steel	Molybdosilicic acid	(*201*)
	Water	Molybdosilicic acid	(*265*)
	Titanium alloys	Molybdenum blue	(*109*)
	Water	Molybdenum blue	(*59*, *420*)
	Biological media	Molybdenum blue	(*312*)
Te	...	Iodide	(*186*)
	...	Thiourea	(*279*, *280*, *456*)
	Magnesium alloys	Sulfuric acid	(*659*)
	Copper-tellurium alloys	Thiourea	(*455*)
	Tellurium-lead alloys	Iodide	(*76*)

tion of cyanide and cyanate. An empirical diagram for determining the nickel and cobalt in sulfate solutions has been suggested (*599*). The simultaneous spectrophotometric determination of calcium and magnesium in water is possible (*689*).

Iron, copper, and manganese are determined successively in organic compounds using diethylammonium diethyldithiocarbamate. The iron is first removed from the digestion mixture by extraction of iron(III) cupferrate with chloroform. Either the cupferrate extract or the diethyldithiocarbamate method can be used to determine the iron. The aqueous phase from the extraction is reduced with sodium pyrosulfite, diethyldithiocarbamate reagent is added, and the copper(I) complex is extracted with chloroform and its absorbance is measured. The manganese is then determined with diethyldithiocarbamate (*672*). Successive determinations of ortho- and pyrophosphate have been achieved by taking advantage of the time required for the pyrophosphate to be converted to the orthophosphate. Cysteine was used to catalyze the pyrophosphate conversion reaction. Thus, by measuring the difference in absorbance of the solution after 7 and 90 minutes using the molybdenum blue method it is possible to determine the amount of pyrophosphate (*158*). Consecutive photometric analysis has also been used to determine the inorganic and organic bound phosphorus in sea water. After determination of the inorganic phosphorus by the molybdenum blue method on the original sample, another sample is oxidized with perchloric acid and the total phosphorus is determined. The amount of organic bound phosphorus is determined by difference (*223*). The determination of phosphorus in the presence of silica by means of successive photometric determinations has also been developed (*153*). The determination of iron and aluminum using 2-picolinic acid as reagent for iron(II) in presence of cyanide and followed by the extraction of the aluminum 8-quinolinol complex with chloro-

Table IV. Photometric Determination of Organic Compounds

Constituent	Material	Method or Reagent	References
Acetaldehyde	...	Schiff	(*54*)
Acetylacetone	...	Ferric alum	(*67*)
	...	Iron(III) chloride	(*586*)
cis-Aconitic acid	...	Naphthol-urea and pyridine	(*433*)
Amino acids	...	Schiff	(*53*)
α-Amino acids	...	Ninhydrin	(*676*)
Arginine	...	Naphthol-urea	(*604*)
	...	Molybdotungstoarsenic acid	(*691*)
Ascorbic acid	Biological fluids	Diazotized 4-methoxy-2-nitroaniline	(*561*)
Aureomycin	...	Molybdoarsenate	(*492*)
Benzanthrone-2,2-dibenzanthonyl	...	Concd. sulfuric acid	(*657*)
Biacetyl	Beer	Nickel dimethylglyoxime	(*656*)
Bilirubin	...	...	(*341*)
Calcium pantothenate	...	Naphthalenediol	(*616*)
Carbamylamine acids	...	Urea-biacetyl reagent	(*324*)
Carbohydrates	...	Calcium resorcinol-4,6-disulfonate	(*364*)
	...	β-Methylindole	(*207*)
	Plant extracts	Anthrone	(*683*)
Carbomycin	...	Molybdoarsenate	(*492*)
Carbon disulfide	...	Molybdate	(*695*)
Carotenoid pigments	Plants	...	(*321*)
Cetylpyridinium chloride	...	Bromocresol purple	(*159*)
Chlorophyll-α and -β	...	Pyridine	(*655*)
Chloropicrin	Air	*N*-(1-Naphthyl)-ethylenediamine dihydrochloride	(*152*)
Cholesterol	Serum	Iron(III) chloride	(*52, 496*)
Choline	...	Hexanitrodiphenylamine	(*551*)
Citric acid	...	Acetic anhydride and pyridine	(*433*)
Coramine	...	Copper(II) sulfate	(*344*)
Cyclohexanol	...	*p*-Hydroxybenzaldehyde–sulfuric acid	(*119*)
Cyclohexanone	...	*p*-Hydroxybenzaldehyde–sulfuric acid	(*119*)
Ethanedial	Biochemical materials	2,3-Diaminophenazine	(*126*)
Ethylenediaminetetraacetic acid	...	Chromium complex	(*102*)
Erythromycin	...	Sulfuric acid	(*160*)
	...	Molybdoarsenate	(*492*)
Fluo-organics	Air	Molybdosilicic acid	(*491*)
Formaldehyde	Cellulose acetate formal	Schiff	(*327*)
Fructose	...	Folin-Denis	(*226*)
	Monosaccharide mixture	Sodium nitroprusside	(*50*)
Galactose	Blood	Orcinol	(*154*)
Gelatin	Plasma, urine	Ninhydrin	(*531*)
Gentistic acid	Blood	Iron(III) gentisate	(*90*)
Gluconic acid	...	Copper glyconate	(*6*)
Glucuronic acid	...	Naphthoresorcinol	(*235*)
Glucose	...	Chlorine dioxide	(*602*)
	...	Phenol and sulfuric acid	(*27*)
	Blood	Orcinol	(*154*)
	Blood	Sulfuric acid	(*397*)
	Blood serum	Chromotropic acid	(*318*)
Glycogen	Tissues	Anthrone and sulfuric acid	(*289, 306*)
Hexamethylenediamine	...	Nitroprusside	(*89*)
Hexoses	Sugar mixtures	Sulfuric acid and thionalide	(*387*)
Hydrazobenzene	...	*N*-1-Naphthylethylenediamine	(*644*)
Hydrocortisone	Ointments	Sulfuric acid–acetic acid	(*617*)
β-Hydroxyethylamines	Surface active agents	Nitroprusside and diethanolamine	(*536*)
o-Hydroxyphenylacetic acid	Penicillin fermentation solutions	Nitrosation	(*481*)
Isonicotinoyl hydrazide	Blood plasma	4-Pyridylpyridinium dichloride	(*509*)
	...	*p*-Dimethylaminobenzaldehyde and isobutyl alcohol	(*309*)
Isovaleraldehyde	Solvent	*p*-Hydroxybenzaldehyde–sulfuric acid	(*119*)
α-Ketoglutaric acid	Blood	2,4-Dinitrohydrazine	(*621*)
4,3-Ketosteroids	...	Tetrazolium blue	(*399*)
	Vegetable oil solutions	Isonicotinic acid hydrazide	(*77*)
Lactic acid	Biological media	Barker-Summerson	(*68*)
Maleic hydrazide	Animal tissue	*p*-Dimethylaminobenzaldehyde	(*670*)
Methanol	Ethanol	Chromotropic acid	(*677*)
Methyl β-(*p*-toluidine) propenyl ketone	...	Iron(III) alum	(*67*)
Methyl bromide air	...	Pyridine	(*359*)
Methylenedioxyl group	...	Chromotropic acid	(*48*)
1-Naphthol	...	Differential reaction rate	(*485*)
Nicotinamide	...	Copper(II) sulfate	(*344*)
Olefins	Air	Modified molybdenum blue	(*373*)
Oxalic acid	...	Indole	(*43*)
Oxidases	Corn root tip	Leuco-2,6-dichlorobenzononeindo-3′-chlorophenol	(*192*)
Phenol	Salicylic acid	Diazotization	(*559*)
Phenol ethers	Surface active agents	Sulfuric acid–formaldehyde	(*535*)
N-Phenyl-1-naphthylamine	Oil	Diazotized *p*-nitroaniline	(*349*)
o-Phenylphenol	Sheepskin	Titanium sulfate	(*92*)
Pine oil (α-terpineol)	Water	Vanillin hydrochloride	(*609*)
1,2-Propylene glycol	Solvent	*p*-Hydroxybenzaldehyde–sulfuric acid	(*119*)
Purine	...	Prostatic acid phosphatase	(*356*)
Pyridine	Water	Barbituric acid	(*12*)
Pyrimidine	...	Prostatic acid phosphatase	(*356*)
Pyruvic acid	Blood	2,4-Dinitrohydrazine	(*621*)
Quinolinic acid	Tissue	Iron(II) quinolinate	(*520*)
Raffinate	...	Permanent standards	(*408*)
Salicyclohydroxamic acid	...	Vanadic acid	(*647*)
Serum acid phosphatase	Prostrate	Tartrate-phenylphosphate	(*155*)
Sorbose	...	Folin-Denis	(*226*)
Sugars	...	Benzidine	(*286*)
Tannins	Fruit	Folin-Denis	(*590*)
Terramycin	...	Molybdoarsenate	(*492*)
Thiamine	Pharmaceuticals	Reineckate	(*30*)
D-γ-Tocopherol	...	Nitrosation	(*505*)
Tyrosine	...	Molybdotungstoarsenic acid	(*691*)
Uronic acids	...	β-Methylindole	(*207*)
Trichloroacetic acid	Plant tissues	...	(*625*)
Vitamin A	...	Perchloric acid	(*117*)
Vitamin B_{12}	Liver extracts	1-Nitroso-2-naphthol	(*182*)

form has also been described (*376*). Iron(II) and copper(I) have been determined by extracting the copper(I) complex formed with 1,10-phenanthroline with octanol, with the iron(II) complex remaining in the aqueous phase (*662*).

Color Specification. Richter (*526*) has explained the official German standard color chart (DIN-Farbenkarte) and pointed out that a complicated relation exists between the Munsell system and the DIN system. The colors are classified according to hue, saturation, and darkness degrees in the DIN system. Middleton (*402*) has reported the results of measuring the colors of paint samples with a normal-diffuse (GE) spectrophotometer and a 45° normal colorimeter. A comparison of instrumental and visual Munsell notations for pyrotechnic smokes showed good agreement for hues and values, but higher chromes were obtained with the special tristimulus colorimeter (*459*). Gibson and Neubrech (*189*) discuss the results obtained with a Munsell meter.

By selecting two chromatic paints from a total of six chromatic paints and mixing with white and black paint, numerous colors can be obtained. The colorimetric specifications of these paint specimens were then correlated with the relative amounts of chromatic paints, black, and white paint. The CIE and Munsell specifications were determined and graphs constructed showing the relation between colorant ratios and color coordinates (*123*).

Bingley (*57*) explained a method by which chromaticity coordinates can be transferred from an arbitrary chromaticity diagram to another chromaticity diagram. The use of a color difference index in comparing small color differences in highly selective transparent colors in acrylic resins has been discussed by Webber and Billmeyer (*653*).

The availability of Munsell charts for the determination of the color of plant tissues and their use in determining the nature of deficiency diseases or the presence of toxic substances has been discussed by Wilde and Voight (*661*).

Special studies include the determination of the yellow color in raw rubber latex films and crepes (*310*), the coloration of dichroic topaz crystals (*346*), and the color of monodispersed colloidal gold (*633*).

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Infrared Spectroscopy

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THIS biennial review has been made critical rather than as comprehensive as the earlier reviews (*17*).

The omission of any article must be taken as reflecting the limited interests of the author of the review rather than the unimportance of the article.

Since the 1954 review, several important meetings have been held both in this country and abroad. An outstanding one was the Gordon Conference on Infrared Spectroscopy held at Meriden, N. H., during the week of August 2, 1954. Another similar conference is being planned for this year and it is hoped that many important foreign infrared spectroscopists will be able to attend. The annual Ohio State University Symposium and the Pittsburgh Conference continued to attract many persons interested in molecular structure and analysis. At least three universities—Massachusetts Institute of Technology, Brooklyn Polytechnic Institute, and Fisk University—held special summer courses on the subject. The European Molecular Spectroscopy Group met at Oxford, July 7 to 11, 1955, and heard over 80 papers.

A number of reviews on infrared spectroscopy were published during the 2-year interval in many journals other than in those appealing to the professional spectroscopist. These have served to acquaint specialists in other fields with the utility of spectroscopy. An outstanding set of reviews on near-infrared spectroscopy (*21*) has been published by Kaye. This region has been neglected since the first quarter of the century and is now again coming into its proper place because of its distinct cell size advantages. The advent of modern instrumentation such as the Beckman DK and Warren Spectracord will enhance the application of this region which fell into disuse when rock salt prisms became generally obtainable. Kaye discusses spectral identification, analytical applications, instrumentation, and techniques with many references to the literature. Anyone contemplating use of this special region must read these reviews.

The books or sections of books published on the subject during the past 2 years include Bellamy's "Infrared Spectra of Complex Molecules" (*4*), which is an excellent survey on the determination of chemical structure. This should be on the desk of every chemist who is interested in the use of infrared spectroscopy as an aid in structure identification. It is not a catalog of spectra, but reviews the literature on spectra-structure correlations.

Mizushima's "Structure of Molecules" (*25*) deals with internal rotation in organic molecules as well as with the principles and experimental methods of structure determination. Normal vibrations are also discussed.

Bak has written an introduction to molecular spectra (*1*) which gives a good treatment of its subject matter for beginning theoretical chemists and physicists, but has little practical spectroscopy for the industrial spectroscopist.

Wilson, Decius, and Cross have written the definitive text on molecular vibrations (*37*), which should be in every spectrographer's library, even though his primary interest may be practical rather than theoretical.

INSTRUMENTATION AND TECHNIQUES

There have been no revolutionary changes in instrumentation during the period. Baird has offered a redesigned spectrophotometer with very rapid prism interchange. Beckman has introduced a relatively inexpensive instrument with slit controls, so that almost per cent transmittance spectra can be obtained. Perkin-Elmer has improved its Model 21 and offered an intensity integrating device for this instrument. In the near future grating instruments using the Merton-Sayce, British National Physical Laboratory, type of inexpensive gratings (*11*) should find wide use (*15*).

A number of papers appeared on the use of the potassium bromide pellett technique for sample preparation. Kirkland (*22*) has made a critical study of the technique in quantitative applications and finds that the precision compares favorably with that in liquid phase techniques. For most qualitative analysis this reviewer feels that the disadvantages of absorbed water or special handling; the consumption of time in disk preparations; and the cost of die replacement, equipment, and optical quality potassium bromide as compared with these factors in mineral oil or hexachlorobutadiene mull techniques make the latter techniques those of choice for 99% of sample preparation.

Two papers worthy of thought and of general interest to all workers in infrared spectroscopy should be noted. The first, by Giese and French (*16*), deals with the analysis of overlapping spectral absorption bands by derivative spectrophotometry. They show that when a band of low intensity overlaps one of higher intensity the detection of the weaker band can be greatly facilitated by measuring the first derivative of the absorption curve *vs.* the wave length. This may be of value in many instances of overlapping.

The second paper offers an interesting solution to the absorbance–optical density nomenclature dispute. Stevens suggests that the unit of attenuation, the decibel, may be used in optics as well as acoustics (*33*). This reviewer has enjoyed the startled looks when he has told organic chemists that their compound had a 7.5-decibel absorption at 1736 $cm.^{-1}$ With the current popularity of hi-fi it should be possible to find more people acquainted with the unit of attenuation than with absorbance or optical density.

An important application of microspectroscopy to double-beam null-type instruments has recently been made by Blout (*5*). Microspectroscopy has been confined mostly to use with single-beam or ratio-recording instruments, but Blout shows that it may easily be adapted for use with the common null instruments.

REFLECTION SPECTRA

Jacquez and his coworkers (*19*) have made a study of integrating spheres for use in the near-infrared. It was found that a combination of coatings allows the measurement of reflectance with an accuracy within 1%. There has been a need for such measurements of diffuse reflectance for some time in the near-infrared, in connection with both medical problems and the plastics field.

SURFACE CHEMISTRY

A number of papers have appeared in the past 2 years showing the application of spectroscopic methods to the study of chemisorption. Eischens and coworkers (*12*) in connection with catalyst studies have studied chemisorbed ammonia and carbon monoxide. Their techniques may be used to advantage by other workers interested in catalysis.

QUALITATIVE ANALYSIS

The interest in the application of infrared spectroscopy to qualitative analysis and determination of structure is certainly increasing. The number of papers is so large and so varied in content that it is impossible to mention them individually. The studies on collections of compounds are also increasing and are of great value both in identification and in spectra-structure correlations. If the journals still accepted spectra for publication, these studies would be of much greater value to practicing spectroscopists. Minkoff, Williams, and Mosher's (*24*, *36*) collection of hydroperoxides and peroxides, Childers and Struthers' (*8*) sodium salts of organic acids, and Ramachandran, Epp, and McConnell's (*31*) phenylhydantoins of amino acids have all been of great utility. Corbridge and Lowe (*10*) have extended the existing work on phosphorus compounds to the inorganic field. The important work of Naves, Lecomte, and coworkers (*27*) in the essential oil field should be available to everyone interested in this class of material. Spectra are used to characterize the compounds obtained from separations and chemical work.

HYDROGEN BONDING—CHELATION

A quantitative approach to the study of this phenomenon has been made by Tsubomura (*35*), who finds that the frequency, shape, and molecular extinction coefficient of the OH bond in phenol are not changed by the concentration of the proton acceptor. Barrow (*3*) has also made extensive measurements of the intensities of hydroxyl bonds. Ferriso (*14*) and Hornig have extensively studied the structure of the oxonium ion, which should be of interest to anyone studying hydrogen bonding. Margoshes and coworkers (*26*) have found in the crystalline state that there is a linear frequency-distance relationship for straight hydrogen bonds, while bent bonds show greater deviation, with the frequencies always higher.

Increased interest in chelation and coordination compounds is shown by the appearance of at least seven papers during the past 2 years. Those interested should look up the work of Sheppard, Bellamy, Martell, Quagliano, Fernelius, and Wiberley.

BIOLOGICAL

Jones (*20*) has summarized 460 absorption bands characteristic of specific molecular structure in steroids, including about a hundred group frequencies recently identified but not previously reported. Cole (*9*) has reviewed the status of spectra-structure correlations in the steroid field very thoroughly. Steroid workers will find great value in these two papers. Thomas and coworkers (*34*) have done interesting work on the examination of nervous tissue and the identification of microorganisms. In the latter case the difficulties of the standardization of the technique, which appears to be of prime importance, are discussed. This reviewer feels that the identification of bacteria by infrared spectroscopy needs much more work.

POLYMERS

Rugg, Smith, and Bacon (*32*) have shown that the structural changes produced in polyethylene by heat oxidation are different from those effected by photo-oxidation. Rugg has found some production of hydroperoxide by heat oxidation as well as the production of different kinds of carbonyl groups.

Elliott (*13*) has continued his excellent work on polypeptides, showing the presence of folded and extended forms through the use of polarized radiation.

The Office of Technical Services, U. S. Department of Commerce, has published an extremely valuable but anonymous bulletin on the spectra of plastics and resins (*28*), which should be in every spectrographic library. The chemistry of the plastics and resins as well as the spectra is discussed in detail.

ABSORPTION INTENSITIES

Thompson (*23*), Crawford (*6*), and their coworkers are publishing the results of their studies on band intensities. Both have shown an interest in bond polar properties in relatively simple molecules and the former has empirically studied homologous series, finding some regularities in more complex molecules of interest to chemists. Gunthard (*18*) has studied intensities in chain molecules.

THEORETICAL AND MOLECULAR

The number of papers published with a theoretical or molecular structure interest is exceedingly large. It is impossible to begin to cover the important papers because of the wide variety of interests of the authors. The work of Hornig (*30*) and Pimentel (*29*) on solids, Lord (*2*) on ring structures, and Sheppard (*7*) on rotational isomerism is among the series of papers that should be especially mentioned. Anyone having an interest in a specific molecule should search the literature in order to find if that molecule has been studied.

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Ultraviolet Spectrophotometry

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THIS biennial review covers the time elapsed since the last review on ultraviolet absorption spectrophotometry by Rosenbaum (*106*) until about the end of October 1955. It is intended to be selective and critical rather than comprehensive, and as a result many worth-while papers on analytical spectrophotometry in the ultraviolet may not be mentioned. Publication of ultraviolet spectra in connection with structural, reaction-rate, and other applications continued at an increasing pace, as did papers of strictly analytical applications. As in past reviews, spectra published in connection with structure-spectra correlations and description of compounds and reactions are not included.

BOOKS AND REVIEWS

A book by Gillam and Stern (*42*) was solely concerned with ultraviolet absorption spectra. This text empirically correlates ultraviolet bands and chemical constitution and presents a large store of information, principally in the form of tabulated data and references. It tends, however, somewhat to slight modern developments, in that considerable emphasis is placed on photographic techniques and that the bibliography extends only to about 1951. Gould (*48*) and Brode (*12*) have reviewed this book.

Several books concerned with instrumental analysis have appeared; these (*9, 35, 54*) generally devote one or two chapters to ultraviolet spectrophotometry, and consequently suffer from this necessary brevity. Considerable emphasis is usually placed on instrumentation rather than on applications.

Volume II of "Radiation Biology" (*111*) which deals with ultraviolet and related radiations contains a great deal of information not elsewhere readily available; the chapter on ultraviolet absorption spectra is particularly valuable.

Reviews on the more theoretical side of ultraviolet spectroscopy (but which should be of considerable worth to the analytical spectroscopist) have been written by Walsh (*132*) and by Sponer (*114*) in the 1954 and 1955 issues of the "Annual Review of Physical Chemistry." A review by Orgel (*95*) covers the little known but significant field of charge-transfer spectra. Badger's book (*6*) devotes a chapter to absorption and fluorescence spectra of aromatic compounds. Gunther and Blinn (*51*) list spectra of important insecticides.

BIBLIOGRAPHIES AND INDEXES

This class of "literature about literature" has its value in keeping the reader informed about articles of interest which appear in publications that he does not ordinarily read. Mellon (*86*) has discussed the literature problem in general. The new British journal, *Analytical Abstracts*, gives comprehensive coverage of analytical articles and excellent abstracts. Articles using spectrophotometry may appear under almost any classification, however, so all categories need to be checked. The British quarterly, *British Bulletin of Spectroscopy*, devotes half its space to molecular spectroscopy, a good share of which is pertinent to analytical applications of ultraviolet spectrophotometry. Prescott (*102*) supplies an index to articles of applied spectroscopy in *Applied Spectroscopy* which lists current publications in absorption spectroscopy but does not include abstracts. Recently, Odeen (*94*) has revised the "Bibliography of Applications of Beckman Spectrophotometers" to include articles up to June 1954. This bibliography covers visible absorption and flame photometry as well as ultraviolet absorption. Indexing is by subject matter, and titles, authors, and journal references are given but no abstracts. Unfortunately, there is no author index.

PUNCHED CARDS

Committee E-13 of the American Society for Testing Materials commenced the distribution of IBM punched cards coding ultraviolet absorption spectra from the literature. The chemical structural coding and other features follow those of the infrared cards whose distribution was started earlier. The codes and instructions for all ASTM-IBM punched cards have been described in detail in a booklet prepared by the committee (*3*). The first issue of cards included 1284 cards, about 600 of which were prepared from spectra which appear in the Friedel and Orchin collection (*39*). A second issue of cards should have appeared by the time of this review, also composed of spectra abstracted from the literature by a corps of volunteer abstractors. The compilation of this collection of spectral data, which includes not only current publications but data back at least to the beginning of the "photoelectric era" (circa 1940), should, within a few years, permit literature searches to be done on IBM machines in very short times, instead of the presently nearly impossible task in the library. This use may well become as important as the originally intended use of identification of unknowns.

INSTRUMENTS AND CELLS

In this country, there are now a number of manufacturers of automatic recording spectrophotometers for use in the ultraviolet region. Applied Physics Corp. (Cary) has added the versatile model 14 to its line; it is an instrument featuring a double monochromator using both a grating and a prism and extending its useful spectral range from the near infrared down to about 1850 A. with a minimum of stray light (*4*). Beckman Instruments, Inc., has added a compact Model DK-2 to its previous DK-1, both instruments using the DU monochromator. Another recording spectrophotometer using the DU monochromator is made by Warren Electronics, Inc., and described by Royer and others (*109*). A universal spectrophotometer is now produced by Perkin-Elmer Corp., described by Coates, Miller, and Savitzky (*22*), using a fused silica prism in a Model 21 infrared instrument with a hydrogen source and photomultiplier detector. Several other recording attachments have been devised for the Beckman DU monochromator, such as that described by Nielsen (*91*). Water-prism monochromators have been built and tested by Fluke and Setlow (*36*).

An interesting variation on means for measuring ultraviolet radiation was presented by Price and Hudson (*103*) by applying a halophosphate fluoroscope and a Geiger tube which was sensitive only to short ultraviolet radiation to the measurement of the absorption of chromatograms. The detector ignored visible and long wave-length ultraviolet and was capable of measuring high absorbances. A double-compartment cell was used by Mitzner and Lewin (*39*) for studying interactions between substances using infrared, but the principle is also applicable to ultraviolet work. Similarly, short path infrared cells were described by Hochheimer and Moore (*63*) which could also be used in the visible or ultraviolet regions. Mitzner (*88*) utilized infrared cells to obtain the ultraviolet spectra of pure liquids, thereby avoiding solvent effects. Another device designed for other spectral regions but usable in the ultraviolet is the reflecting objective for microspectrophotometry of Thornburg (*122*).

A versatile low- or high-temperature cell for the Cary spectrophotometer was described by Geiger (*40*); Hamner, Hadden, and Padgett (*53*) devised a 50-cm. heated gas cell for use with the Cary. Inserted materials have been used to reduce volumes of Beckman cuvettes (*113*). A "dry calibration" technique for absorption cells was advocated by Archer (*5*). Wyman (*137*) successfully applied potassium bromide disks to obtaining ultraviolet spectra with the Cary spectrophotometer by use of a special disk holder. Vandenbelt and others (*128*) showed that there is a constituent of human perspiration with strong ultraviolet absorption which could cause errors if deposited on cell windows.

The newest recording spectrophotometers (Cary 14, Beckman DK-1 and DK-2, Spectracord, and Perkin-Elmer Universal) are too recent for comparative studies to have appeared. However, two intercomparison studies of Cary Model 11 spectrophotometers were carried out by volunteers from members of the American Drug Manufacturers Association. The first of these, described by Vandenbelt (*127*), used calibrated glass filters for intercomparison in the visible region, while the second (*131*) used chromate solutions in the ultraviolet. Very good agreement was found among the instruments. Another study by Ketelaar and others (*72*) covered one Cary and one Zeiss and several Beckman instruments; good agreement was found, even though the slit widths used varied widely among the various instruments. Tarrant (*118*) described the operation and performance of a Cary spectrophotometer at the National Physical Laboratory (England). Most recently, Cahn (*15*) presented an article on photometric reproducibility between ultraviolet spectrophotometers, which was largely a comparison of the Cary 11 and Beckman DU instruments. While avoiding the current controversy between the proponents and opponents of differential spectrophotometry, Cahn presented some significant data and comments regarding the real precision of spectrophotometric measurements by conventional means.

BEER'S LAW

The law of the absorption of electromagnetic energy, generally called Beer's law, which is the basis of quantitative spectrophotometry, was given a variety of usual and unusual applications. Perhaps fewer alleged "deviations" from this law have found their way into print these two years because, it is hoped, of a better awareness of the validity of this law and of the "apparent" deviations due to scattered light, fluorescence, instrumental and operational defects, and errors in counting the number of absorbing species present. A valuable contribution to the interpretation of absorption curves which result from overlapping bands was made by Vandenbelt and Henrich (*129*), who presented plots of idealized curves which had been added together; these covered a broad range of relative band intensities and separations. Most recently, Giese and French (*41*) have offered a method of analyzing such overlapped absorption curves by plotting the first derivative of absorbance as a function of wave length. Several graphical illustrations were presented and an instrumental method of plotting was promised; considerable experimental evaluation is needed.

An "absorbance-ratio" method for ease in setting up two- and three-component analyses was described (*58*). Ross (*108*) discussed Beer's law for conditions of samples of nonuniform concentration and of irradiated glasses. The temperature dependence of absorbance was studied by Yarborough, Haskin, and Lambkin (*138*).

Stray light originating from internal reflections in prisms was discussed (*32*). Tunnicliff (*124*) presented a study of the measurement of nearby stray light in ultraviolet spectrophotometers, in contrast to the usual general stray light studies. For this, the extremely sharp absorption of the 2537 A. line by heated mercury vapor in a sealed cell was utilized. Interest in the experimental measurement of stray light in modern spectrophotometers is most encouraging in view of the tendency to make use of high absorbance readings and to use high absorbance blanks. Measurements are being pushed to the lowest possible wave length limits, especially with the advent of fused silica optics having better transmittance at very short wave lengths (used in several new instruments).

A most interesting experiment, which could be tried by everyone owning any kind of spectrophotometer, is that of Mehler (*85*), who used a series of samples prepared to have absorbances of 1, 2, 3, 4, and 5 at a particular wave length. These were examined successively, starting with the $A = 1$ using a solvent blank and using each sample with its preceding sample as a blank; thus each sample should have given an instrumental reading of 1 absorbance unit. The results are something that every advocate of using high absorbance blanks in differential spectrophotometry must consider.

Attention should also be called to a brief (but most pertinent) editorial on the topic of reproducibility (*101*). Two articles on the fluorescence error in spectrophotometry, one by Ovenston (*96*) and another by Braude and Timmons (*10*), are important. More recently, Gridgeman (*50*) presented a critical discussion on precision and errors in differential spectrometry, and Cannon (*17*) reported an anomalous error encountered in this technique, whereby the absorptivity appeared to increase rather than decrease with slit width. Reilley and Crawford (*104*) discuss the principles of precision colorimetry and some possible errors. An unusual variation of the differential method, presented by Lothe (*80*), is called indirect differential spectrophotometry; this was published rather too recently for experimental evaluations or criticisms to appear.

SPECTROPHOTOMETRIC TITRATIONS

Recording ultraviolet spectra as a function of pH or of amount of titrating solution added may be used to determine ionization constants, to perform quantitative analyses for the material being titrated, or to make use of an absorbing material as an "ultraviolet indicator" for the titration of some transparent material. Several devices for making these operations easier and faster have been described. A "spectrotitrimeter" (*73*), using a glass-piston pump to circulate the solution through the absorption cell of a Cary spectrophotometer and titration vessel, included a thermostating arrangement and displacement of air with an inert gas. Fricker (*38*) described a somewhat simpler device which uses a high-speed glass centrifugal pump to circulate the solution about a compact system. This apparatus was for use in visible spectrophotometry, but with quartz cells it would be applicable to ultraviolet as well. A titration assembly (*75*) for a Beckman DU used a magnetic stirrer within a titration vessel which was also the absorption cell.

A comparison of pH values determined by electrometric titration and by ultraviolet absorption methods showed good agreement between the two methods (*130*). A method for calculating dissociation constants from spectrophotometric data has been described by Rosenblatt (*107*), which is applicable where it is impossible to determine absorptivities of the pure ionic or molecular forms. Thamer (*121*) described means of determining overlapping pK_a values of dibasic acids which did not require direct knowledge of the individual ionic forms. Irving, Rassotti, and Harris (*67*) discussed extensively a general method for calculating pK$_a$ values based on points of inflection of a pH *vs.* absorbance curve. A chart of pK ranges for various ionizable organic groups was presented along with a discussion of the use of apparent dissociation constants in qualitative organic analysis (*98*). A review (*44*) on photometric titrations contained 105 references and was devoted largely to visible spectrophotometry but also covered ultraviolet applications. Extension to photometric titrations of nonaqueous solvents (*105*) was made with glacial acetic acid as the medium. Use of a typical "ultraviolet indicator" was made (*82*) in the automatic titration of thorium, using copper Versenate and a Cary spectrophotometer plotting absorbance *vs.* milliliters of titrant.

VACUUM ULTRAVIOLET

Although the vacuum ultraviolet region is not at present considered an important region for quantitative analysis, as most commercial instruments are capable of accurate measurements to only about 2200 A., it is expected to become important soon. Inn (*65*) has reviewed the literature on this spectral region, and Jones and Taylor (*69*) have summarized data in the 1700 to 2200 A. region and term this region a "promising new tool for the analyst." Klevens and Platt (*74*) collected their measurements and some of others into the largest compendium of vacuum ultraviolet spectra extant. The wave-length "guideposts" from 1940 A. down to 875 A. have been mapped in a paper on provisional wave-length standards in the vacuum ultraviolet by Wilkinson (*135*). New photon counters for use in this spectral region have been described by Chubb and Friedman (*20*). A novel instrument, measuring down to 1500 A. (*52*) features a fluorite prism which is vibrated about a horizontal axis while scanning about a vertical axis in order to minimize scattered light effects. A new xenon arc has been described as a source for the vacuum ultraviolet (*136*).

INORGANIC ANALYSIS

Some trends in the application of ultraviolet spectrophotometry seem apparent, such as a tendency to choose a stronger ultraviolet band for the analytical wave length rather than a visible wave-length band in order to obtain better sensitivity (lower detectability); this is sometimes referred to by the term "ultraviolet colorimetry" (which is an unhappy mongrelization of terms to many spectroscopists). There is also a trend toward using complexing agents, notably ethylenediaminetetraacetic acid [(ethylenedinitrilo)tetraacetic acid, EDTA]. Buck, Singhadeja, and Rogers (*14*) have summarized common anions which absorb ultraviolet themselves or as complexes with metals. Determinations of arsenic, phosphorus, and silicon (*27*) and of soluble silicates (*26*) are reported. Niobium has been determined directly in hydrochloric acid (*70*), as thiocyanate complex (*55*), and as a derivative of 8-quinolinol (*71*). Tellurium sols (*68*) are determined at 280 mμ in preference to a visible band because of the variation of the latter with particle size; telluric acid is determined directly (*112*). Pyrogallol was used to determine tantalum (*29*) and to determine tantalum and niobium in ores (*84*). Palladium (*18*), mercury (*83*), and vanadium (*47*) are other examples.

For EDTA complexes, a sampling of the literature showed a summary for five elements (*117*), bismuth determined near 400 mμ (*19, 125, 134*), and determinations of iron (*90, 126*). One of these procedures for iron (*90*) was developed as a microprocedure claimed to be good down to 4 p.p.m. Many other metal determinations by complexing agents and spectrophotometry have been reported; an interesting application of these is as "ultraviolet indicators" for titration performed to determine some other substance. An indirect method was applied to the determination of small amounts of oxygen in water, by making use of the strong absorption of the triiodide ion at 353 mμ (*97*). For analyzing oxides of nitrogen, nitrogen dioxide and nitrogen tetroxide are determined at 394 mμ and the other components by mass spectrometry (*92*).

ORGANIC ANALYSIS

In viewing the tremendous growth which has taken place in the field of ultraviolet spectrophotometric determination of organic compounds, one is impressed with the trend to employ solvent-solvent extractions, precipitations, complexing agents, chemical separations, or chromatography as an inherent part of the analytical scheme. These serve to rid the material to be analyzed of substances which have overlapping absorptions, to concentrate the desired material to obtain a lower detectability, or generally to make a simple analysis out of a hopelessly complex one. Many analytical schemes are now dependent upon chemical solvent or chromatographic separations prior to spectrophotometric identification or quantitative analysis. In some applications the change in observed spectra with changes in pH or solvent may be utilized. Some of the following examples may be used as illustrations.

By the use of selective solvent extractions, curing agents in rubber products may be identified (*76*). Lacquers may have nitrocellulose and phthalate esters analyzed (*116*). Extraction permitted the determination of nicotinic acid in tobacco leaves (*37*) and the conjugated acid content of milk fat (*77*), for example. Separations prior to spectrophotometric analysis permitted the determination of alkaloids in opium (*31*). Accelerators and antioxidants in rubber products were determined by Brock and Louth (*11*).

In recent years, the natural cooperation between spectrophotometry and chromatography has changed from the use of the spectrophotometric methods for the monitoring of chromatographic column effluents into the incorporation of chromatographic separations as intrinsic parts of analytical schemes to effect separations prior to spectrophotometric examinations. While the analysis of column effluent will continue to be an important application of ultraviolet spectrophotometry, the preliminary separations will become increasingly important to the analyst. Some examples of this include the detection of antioxidants in rubber stocks (*62*), theobromine and caffeine in cocoa (*33*), pyrethin synergists (*8*), vitamins A and D (*13, 34, 79*), mixtures of phenols (*99*), and α-keto acids in blood and urine (*119*). Such separations were discussed by Crowe and Walker (*24*). Cooper (*23*) applied chromatographic separations to the determinations of polycyclic hydrocarbons in city air. A similar application to effluents from gasworks was made (*133*).

Some examples of spectral shifts with pH which were used in analytical schemes were the work of Goldschmid (*46*) on lignin, the distinction of morphine from codeine (*21*), Meyer's investigations on steroids (*87*), and the determination of erythromycin by application of alkaline hydrolysis and an acidic blank (*120*). The acid hydrolysis of melamine resins to melamine at reflux temperatures was used in the analysis of wet-strength paper (*59*). Shifting the pH of the solution being examined permitted the determination of tetracycline, oxytetracycline, and chlortetracycline at three wave lengths and three pH values (*66*). The shift of the spectra of phenols with variations in pH was combined with a chloroform separation in analyzing waste waters (*110*). A similar use of pH shifts of absorption spectra was employed by Goldman (*45*) in determining nicotinic acid hydrazid.

Ahlers and O'Neill (*1*) reviewed the structure of oils and resins as determined by spectroscopy. Stafford, Shay, and Francel (*115*) presented the infrared and ultraviolet absorption spectra of the more important dicarboxylic acids for purposes of identification in the analysis of resins. Using the Kappelmeier separation method, as modified by the American Society for Testing Materials (*2*), styrenated fatty acids and alkyd resins were analyzed spectrophotometrically (*61*). Herb (*57*) reviewed the application of ultraviolet spectrophotometry to the analysis of fats, giving 56 references. Davis and Bowen (*25*) combined a titration with spectrophotometry to effect the analysis of benzoate and phthalate esters on military clothing. Aromatic sulfonates were determined following an acid-ether extraction in analyzing synthetic detergents (*64*). Total and primary hydroxyl were determined (*81*) in cellulose by the use of reactions to produce ultraviolet-absorbing substances. Styrene and phthalate esters were determined in polyester resins (*60*) and fumarate and glycols estimated.

Complexes with iodine were effectively employed in the analysis of aliphatic sulfides (*30, 56*). Long and Neuzel (*78*) demonstrated that the observed absorbance of iodine complexes with olefins was proportional to the product of the iodine and olefin concentrations. They showed that the wave-length position of the band varied with the number of substituents about the carbon atoms of the olefinic double bond. A large table of examples was presented. The weak absorption of the ketone group near 290 mμ was exploited in the determination of combined methyl isopropenyl ketone in polymers by Pepe, Kniel, and Czuha (*100*).

MISCELLANEOUS

Installation of a Beckman DUR for automatic control in a synthetic rubber plant was described (*16*). Ballard and coworkers (*7*) showed that corrections could be made for interferences from absorbing organic substances in a Beckman DU used as a 2537 A. gas analyzer because of the sharp absorption line of mercury in contrast to the broad organic adsorption bands. Glasser (*43*) described an automatic ultraviolet gas analyzer with a variety of filter combinations to change the effective analytical wave length in order to provide a variety of possible analyses. Application to continuous analysis of gas streams was made. Troy (*123*) used a portable gas analyzer for measurement of atmospheric pollution; this device is now commercially available and should be most useful.

The purification of fluorocarbon liquids was followed spectrophotometrically by Grafstein (*49*); this suggests possible use of these compounds as solvents for ultraviolet spectrophotometry. Data on conjugated terpene hydrocarbons presented by O'Connor and Goldblatt (*93*) should prove valuable for setting up analytical schemes.

The protection of light-sensitive pharmaceutical preparations is discussed by Dimbleby (*28*) and transmittance curves for a number of colored glasses used for bottles are presented, with data extending down to about 300 mμ.

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

X-Ray Absorption and Emission

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THE popularity of analytical methods based upon x-ray emission and absorption, and upon the analogous γ-ray processes, continues to grow. The meetings during the past 2 years at which such methods were discussed are too numerous to list. Abstracts of the Pittsburgh Conference papers (*6–8*) provide a fair index to progress in this field and to the material presented at other conferences. The present review will be easier to follow with these abstracts and the earlier reviews (*88–93*) at hand; references to the individual abstracts are not in general made here.

An event of first importance to all those interested in using x-rays is the appearance, considerably expanded in its fourth edition, of Clark's "Applied X-Rays" (*31*).

In the present review, two important topics—detectors and histochemical analysis—have been given more extended treatment than the others. An attempt has been made to present enough about the absorption and emission of γ-rays to inform the analytical chemist usually concerned with what happens outside the nucleus. The bibliography falls far short of including all the references, especially all the γ-ray references, compiled in preparing the review.

DETECTORS

The subject of x-ray and γ-ray detection belongs primarily to the physicist. The analytical chemist must take notice of it, however, because the detectors concerned are now among the tools of his profession. Were it not for the great improvements that have occurred in these detectors during the past 15 years, there would be little or no occasion for these reviews.

The information about detectors in Table I is perhaps a reasonable minimum for the analytical chemist; further information is available (*14, 46, 50, 75, 131, 143*).

The photographic plate, though invaluable for specialized

Table I. Characteristics of Electrical Detectors

Name	Type	Quantitative	Counting (Time Resolution)	Energy Resolution
Ionization chamber (current)	Gas-filled (low field)	Yes	No	No
Proportional counter	Gas-filled (intermediate field)	Yes	Yes	Good
Geiger counter	Gas-filled (high field)	Yes	Yes	No
Scintillation counter	Photoelectric (phototube multiplier)	Yes	Yes	Moderate
Photoelectric detector	Photoelectric (phototube multiplier)	Yes	No	No
Cadmium sulfide	Photoconducting	No	No	No

Table II. Data for X-Ray Detectors in Figure 1 (*131*)

Counter Number	Counter Type	Absorbing Medium Pressure, Cm. Hg	Active Length, Cm.	Window Thickness, Cm.
1	Geiger, end-window[a]	A, 55	10.0	Mica 0.0013
2	Proportional, side-window	Xe, 30	.2.7	Be 0.0127
3	Proportional, side-window	Xe, 30	2.7	Mica 0.0013 + Be 0.0127
4	Proportional, side-window	Kr, 50	2.7	Mica 0.0013 + Be 0.0127
5	Scintillation	NaI·Tl	0.70	Be 0.0127
6	Scintillation	NaI·Tl	0.10	Be 0.0127
7	Scintillation	NaI·Tl	0.05	Be 0.0127

[a] Norelco, Type 62019, has inactive length about 0.3 cm. included in calculations.

applications (*97*), has not been included because it will scarcely be used when another detector will serve.

The first three detectors (*143*) all depend upon ionization by photons absorbed by a gas in an electric field. The field may be between a positively charged central wire and a coaxial metal cylinder. As the field is increased, there occurs first a region in which the charged particles produced are collected without multiplication (ionization chamber); then a region in which there is significant multiplication, and the number of particles collected is proportional under proper conditions to the energy of the photons (proportional counter); and finally a region of complete gaseous breakdown in which there is enormous multiplication that bears no relation to the energy of the incident photons (Geiger counter). With the improved means of external amplification now available, the current ionization chamber (as distinct from the counting or pulse ionization chamber) deserves to be considered by the analytical chemist for measuring the intensity of relatively strong polychromatic beams (*48*).

Geiger counters, though well known and easy to use, are subject to one limitation (*143*), sometimes serious, which does not hamper proportional and scintillation counters. In Geiger counters, the act of ionization by a single photon produces a sheath of positive ions that reduces the field at the central wire below the potential required to start a discharge by a subsequent photon. This condition will continue for a time—called the "dead time" of the counter—until the positive ion sheath has drifted far enough from the wire to raise the field at the wire above the starting potential. In other words, owing to the phenomenon of dead time, Geiger counter readings will increase less than proportionally to the intensity of the x-ray beam, and the counter will "choke" if the intensity becomes high enough. In addition to the loss of counts by the mechanism just described, all counters are of course subject to additional losses if the resolving power of the recording equipment is inadequate. Counting losses in analytical work are more likely to cause concern with x- than with γ-rays because the former are likely to be used at the higher intensities.

Proportional counters (*63*, *118*) hold promise of great future development in analytical chemistry because they are capable of energy resolution, or "pulse height discrimination" as it is often called, pulse height and energy being proportional in the ideal case. As a consequence, the proportional counter offers a highly convenient means of spectral analysis that does not entail the great sacrifice of intensity concomitant with the use of crystal monochromators for this purpose. To be sure, the resolution possible with crystals greatly exceeds that of which the proportional counter is capable. *Fortunately*, however, the analytical chemist in *general requires* only that the resolution be good enough not to decrease the reliability of his results. The proportional counter, and on occasion even the scintillation counter, can sometimes meet this usually modest requirement.

The modern scintillation counter (*46*, *77*, *100*), which Birks in his monograph (*14*) calls "the most important and versatile instrument in nuclear physics research," uses a photomultiplier tube to count the scintillations produced when x-rays or γ-rays are absorbed by a phosphor such as a thallium-activated sodium-iodide crystal. Its energy resolution is about 2.5 times lower than that of the proportional counter, but it has important compensating advantages (*131*). Perhaps its most serious disadvantage is that unavoidable presence of photomultiplier tube noise limits its usefulness in the region of wave lengths longer than about 3A.

An excellent comparative study of Geiger counters, proportional counters, and scintillation detectors has recently been carried out by Taylor and Parrish (*131*).

Since the detector used has a marked effect on the quality and precision of the x-ray patterns, the x-ray analyst must have a working knowledge of the detector characteristics in order to choose the detector best suited for the problem. There are many factors, such as the linearity of response, quantum-counting efficiency, stability, background, possibility of pulse-height discrimination, etc., which must be considered in choosing a detector for a particular problem for no one detector is ideal for all x-ray problems.

In this paper one of the most important factors, the quantum-counting efficiency, will be discussed. The following example will illustrate its importance. Frequently MoKα radiation is used to obtain single-crystal diffraction patterns. Since the quantum counting efficiency of a NaI·Tl scintillation counter is about 99% and an argon-filled Geiger counter only 13% at 0.71 A., a gain of more than a factor of 7 in the measured intensity would be realized without increasing the power input to the x-ray tube simply by using the former. This is nearly the same

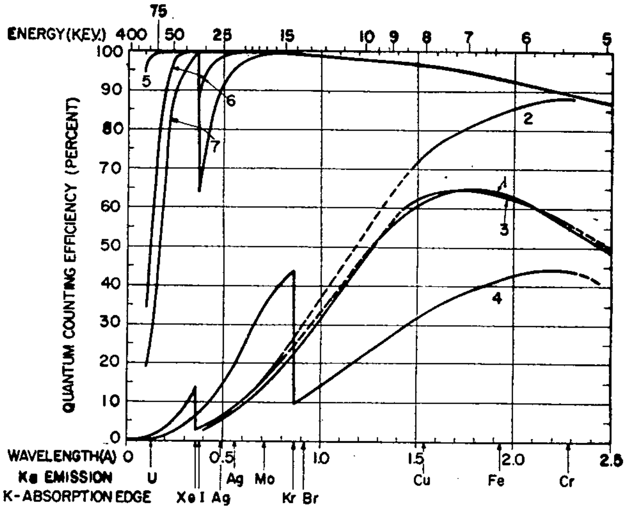

Figure 1. Calculated counting efficiencies of detectors listed in Table II (*131*)

gain in intensity that would be realized in practice by using a high-power rotating-anode x-ray tube, with its additional cost and maintenance difficulties, instead of a stationary-anode water-cooled tube.

The point made above regarding quantum-counting efficiency is clear from Figure 1 and Table II, which contain some of the more important data given by Taylor and Parrish.

Inasmuch as the properties of the window on the counter help determine the efficiency, the type of window is given for each detector. For example, growing absorption by the beryllium window is responsible for the gradual reduction in efficiency of the scintillation counter with increasing wave length. Data for such counters are given for three thicknesses (0.70, 0.10, and 0.05 cm.) of sodium iodide; the figure shows that only the thickest crystal escapes a reduction of efficiency at 0.375 A. owing to the presence of the *K* absorption edge of iodine.

Kohler and Parrish (*75*) have used some of the counters of Figure 1 in the x-ray diffractometry of radioactive samples. Though radioactive samples are growing rapidly in importance, the point of principal immediate interest here is the use of energy resolution (pulse-height discrimination) to separate the radioactive background from the x-ray lines under investigation. The following quotation describes the detection system, which has the important and desirable property of conveniently accommodating any one of several detectors.

> The photomultiplier tube, crystal, and cathode follower comprise a unit which may be called the scintillation head. It is used as a unit and is mounted on the goniometer arm in place of the usual Geiger tube. The cathode follower feeds a linear amplifier with a gain adjustable up to 2500. The amplifier in turn feeds a pulse height discriminator which may be set to pass all pulses greater than a given amplitude (base) or only those pulses being within a specified amplitude range (window). From the discriminator the signal goes to standard scaling and counting equipment. When making measurements such as are shown in Figure 1 (*75*), it is convenient to use a recording counting-rate meter synchronized with a motor-driven base line on the discriminator. An oscilloscope showing the output of the amplifier is useful for monitoring purposes.
>
> When using the proportional counter, the same equipment was used by simply replacing the scintillation head with an equivalent proportional counter head.
>
> The Geiger counter fed the counting equipment directly without the intervening additional amplifier and discriminator.

From the discussion of γ-ray absorptiometry (below), it is clear that the mass absorption coefficients involved are often much smaller than those of the usual x-rays. Consequently, a γ-ray detector to be efficient must often contain a relatively large absorbing mass. Only the scintillation counter, which can accommodate a massive crystal, can conveniently provide such a mass (*49*). It is therefore a powerful γ-ray detector.

Earlier discussions of the photoelectric detector (*88*) suffice for the present. A previous discussion of the cadmium sulfide detector (*93*) needs supplementing in one respect. The small size of this detector makes it well adapted to dosimetric measurements in radiotherapy, and the results obtained have been rather more reliable than might have been anticipated (*20, 51, 124*).

ABSORPTION

For the sake of simplicity, "x-ray absorption spectrometry" has been abandoned as a subdivision in these reviews. The term was used mainly to describe the method of Glocker and Frohnmayer (*53*), which can logically be included under "absorptiometry with monochromatic beams" because the method is usually a differential measurement of this kind. "Absorptiometry with monochromatic beams" is operational, as is desirable, and it connotes "spectrometry."

Absorptiometry with Monochromatic Beams. In the absorptiometry of gases, x-rays of long-wave length must often be used to ensure appreciable absorption. The filtering effect of the sample on a polychromatic beam then often leads to a pronounced decrease in effective wave length (*95*)—an undesirable complication. The development of a gas analyzer that employs a monochromatic beam and a Geiger counter is therefore welcome (*117*).

Another development that is welcome for this and other reasons is the use of radioactive isotopes as x-ray sources (*86, 114*). These must not be confused with γ-ray sources (discussed later), which are also radioactive but in which the transformation leading to radiation occurs within the nucleus. The x-rays from radioactive sources may for present purposes be regarded as monochromatic.

In the radioactive x-ray sources of interest here, the following sequence of events may be thought to occur. The nucleus captures an electron from the *K*- shell, and this transmutes the element into the one next nearer hydrogen in the periodic table. The *K*- shell vacancy in the new element is now filled by an *L*- electron, whereupon a *K* line of the new element is radiated. Although the intensity of this *K* line is usually low, it is no different for purposes of analysis from the same line generated at the same intensity in any other way, but the use of radioactive x-ray sources can nevertheless bring important advantages.

Hughes and Wilczewski (*64*) capitalize on these advantages by using iron-55 as a source for the *K*-lines of manganese for the determination of sulfur in hydrocarbons. The following quotation, of value as a general guide, explains their selection of this radioactive isotope.

> Of the more than 100 isotopes engaged in this process, only iron-55, nickel-59, rhodium-102, and cadmium-109 showed any promise of being useful for x-ray absorption analysis at the time this work was started. The criteria are: The half life should be reasonably long, so that the source does not decay too rapidly, yet not so long that the specific activity is low; no other competing radiation should be emitted (positive and negative β-rays being easily absorbed are excepted); and it should be available and readily processed into a useful, easily handled source. Iron-55 was chosen as the isotope most nearly meeting these requirements.

The experimental method consists essentially of using a Geiger counter to measure the attenuation by the sample of x-rays (wave length 2 A.) from the radioactive source. The usual considerations apply, and the (slow) decay of the radioactive source must be taken into account. The great value of the method is clear from Table III.

The over-all reliability of the method is estimated at 2σ, or 0.05% sulfur.

An important application of the method of Glocker and Frohnmayer (*53*) is discussed in the section captioned "Histochemical Analysis."

Absorptiometry with Polychromatic Beams. Titus (*134*) gives in comprehensive detail the quantitative analysis, by absorptiometry with polychromatic beams, of treated carbon brushes used in aircraft, this being the "point-to-point exploration of

Table III. Comparison of Speed, Accuracy, and Cost of Determination of Sulfur in Petroleum Products (*64*)

	Speed				Relative Cost	
Method	Elapsed time, hours	Operator time, min.	Samples per day	Accuracy Sulfur, %	Equipment	Operator-time
Bomb-sulfur	24	36	13	0.03–0.14	5	7.2
Lamp-sulfur	3	29	16	0.03	1	5.8
Old x-ray method (*65*)	0.12	7	48	0.09	20	1.4
K-capture	0.08	5	60	0.05	6	1.0

impregnated materials" of an earlier review (*135*). The treatment consists usually of impregnating the brush stock with a solution of a salt—e.g., lead chloride, cadmium chloride, barium fluoride, barium bromide—to an extent that will leave the desired amount, usually a few per cent, of salt in the brush when the water has been removed. The purpose of the treatment is to reduce or eliminate excessive wear of brushes used on motors and generators operating at high altitudes. The speed and nondestructive character of the method, and its adaptability to point-to-point exploration, were exceptionally useful in this application.

Grohse (*55*) has found absorptiometry with polychromatic beams to be a unique method for studying the "fluidization" of a finely divided solid by a gas, as in beds of silicon fluidized by air. Bed density profiles, which reveal the character and effectiveness of fluidization, could be readily determined without disturbing the bed—impossible when internal probes are used.

The usefulness of absorptiometry with polychromatic beams in the atomic energy program (*93*) has been extended by Lambert (*30, 79–83, 87*), who applied this method to aqueous solutions of 35 compounds and ions of 25 elements widely distributed in the periodic table. With elements of atomic numbers 42 to 65, Lambert encountered the "deviations from linearity" (*96*) to be expected under certain conditions when an absorption edge falls within the wave-length range of a polychromatic beam. Lambert's extensive and precise absorbance data greatly facilitate x-ray absorptiometry with polychromatic beams.

Terrell and Davidson (*132*) have corroborated published favorable experience (*21, 108, 141*) with the General Electric x-ray photometer in the petroleum industry, and have emphasized particularly the increase in operating efficiency that results because the speed of this x-ray method reduces the time during which the product being tested must remain in compounding facilities or in storage.

Cranston, Matthews, and Evans (*35*) have published the results of an extensive British study of the determination of sulfur in hydrocarbons. The method in principle closely resembles that of the previous paragraph but the equipment is different. The extensive analytical results in general show that the Sunbury method is highly reliable.

Exceptionally interesting, but outside conventional analytical chemistry, is the use of absorptiometry with polychromatic beams to study detonation waves (*74*) and boundary layers (*142*), as is the general subject of x-ray flash photography (*47*).

γ-Ray Absorptiometry. Because γ-rays are formed in nuclear processes, which generally involve high energies, one might expect their wave lengths to be shorter than those of the usual x-rays, which result from electron transitions within atoms. One might expect further, considering the pronounced decrease of x-ray mass absorption coefficients with decreasing wave length, that mass absorption coefficients for γ-rays will be appreciably smaller than those ordinarily met with in x-ray absorptiometry. Table IV, which has been constructed from data in standard references (*60, 114*), shows that both expectations are realized. Further, γ-ray coefficients show markedly the smaller variation from element to element, and this gives x-rays an advantage for applications other than simple thickness gaging.

Of course, the situation is considerably more complex than the simple statements above might indicate. For example, it is well known that scattering and photoelectric absorption both contribute to x-ray absorption coefficients, and that the photoelectric contribution tends to increase with atomic number. As the wave length decreases, the photoelectric contribution becomes markedly less important, and this is the main reason why γ-ray coefficients are so much the smaller. On the other hand, these coefficients include γ-rays consumed in the production (at energies above 1 m.e.v.) of positron-electron pairs, a process impossible with ordinary x-rays. An excellent discussion of γ-ray absorption is given by Bethe and Ashkin (*121*).

Extensive values of γ-ray absorption coefficients are available from both theoretical (*44, 125*) and experimental work, much of the latter being necessary in connection with the shielding problems that have arisen in this atomic age (*9, 45, 54, 57, 85, 107*).

As Table IV makes clear, samples for γ-ray absorptiometry will generally have to be thicker than were x-rays being used. Accordingly, it is not surprising to find that the γ-ray technique is making rapid progress in nondestructive testing, particularly in thickness gaging and in radiography (*5, 18, 22, 52, 67, 140*). The exceedingly high precision (0.01%) attained by Berman and Harris (*12*) in thickness measurements with a cobalt-60 source makes their work worthy of special mention. Many more references could be cited.

Aebersold (*5*) lists as in Table V the radioactive isotopes most useful as γ-ray sources in radiography. These are likely also to be the most useful of the isotopes now available for absorptiometry in general.

Table IV. Absorption of X-Rays and Gamma-Rays

Energy, M.e.v.	Wave Length, A.	Mass Absorption Coefficient[a]				
		Carbon	Water	Aluminum	Copper	Lead
0.01	1.24	2.5	5.2	27	230	138
0.025	0.496	0.31	0.51	1.9	18	53
0.05	0.248	0.18	0.22	0.37	2.8	8.8
0.10	0.124	0.15	0.17	0.18	0.49	5.7
0.25	0.050	0.116	0.124	0.107	0.102	...
0.50	0.025	0.089	0.095	0.081	0.078	0.15
0.75	0.017	0.076	...	0.070	0.065	...
1.00	0.012	0.067	0.069	0.059	0.056	0.071

[a] For definition of mass absorption coefficient, see, for example, (*31, 34, 88*).

Table V. Isotopes for Radiography (5)

Isotope	Half Life	Principal Gamma Ray Energies, M.e.v.	Approximate Specific Activity	Approx. γ-Ray Output/Curie, R/Hr./Ft.[a]	Type of Use
Cobalt 60	5.3 years	1.1, 1.3	35 C/g—1954	14.4	Heavy castings, thick weldments
Tantalum 182	117 days	1.1, 1.2	1.5 C/g	6.7	Heavy castings, thick weldments
Cesium 137	37 years	0.66	20 C/g—1955	3.5	
Iridium 192	75 days	0.3, 0.5	30 C/g—1954	3.0	Pipe lines, light alloys, thin sections
Thulium 170	127 days	0.085	Service irradiation	...	Light alloys, thin sections

[a] Roentgens in 1 hour at 1-foot distance from source.

Leboeuf, Miller, and Connally (*87*) have carried out an extensive investigation of γ-ray absorptiometry with several sources (notably an americium-plutonium mixture) not listed in Table V. These authors were in an exceptionally favorable position to compare γ-ray and x-ray absorptiometry on highly absorbing samples (usually high both in mass and in atomic number). The following quotation summarizes their comparison.

> The principal advantages of using radioisotopes as gamma photon sources are the following:
>
> 1. Extreme stability of source intensity
> 2. Decreased instrument complexity
> 3. Compact size

4. More nearly monoenergetic characteristic of the emitted photons.
5. Much wider range of photon energies available, about 20–2,000 k.e.v.
6. Lower over-all cost of the instrument.

The principal limitation is the low intensity generally available compared to the extremely high intensities from x-ray tubes. Because of this, these sources cannot effectively compete with x-ray photometry in the energy range up to 50 k.e.v. In the range 50 to 400 k.e.v., however, these sources offer attractive possibilities.

Bernhard and Chasek (*13*) applied γ-ray absorptiometry to the measurement of soil density with encouraging results. Further applications should be forthcoming soon.

EMISSION

X-Ray Emission Spectrography. A brief review of this rapidly developing field cannot do justice to all the good work that is being done. It is unfortunately necessary to call attention to the continuing confusion in nomenclature, which is obvious among the 35 papers on the subject given at the recent Pittsburgh Conferences (*6–8*)—especially among those in 1955.

"X-ray emission spectrography" was chosen as a name here because it is operational (a spectro*graph*, not a spectro*scope* is used), and because it includes characteristic x-ray spectra excited by any means whatever—not "fluorescent" x-rays alone.

Especially with x-ray excitation, pronounced "deviations due to absorption" (*19*) can occur, and the intensity of an emitted line consequently cannot be regarded as proportional to the concentration of an element in a sample. These deviations can be due to one or more of the following causes. "Filtering" effect on an incident polychromatic beam, absorption of the incident beam, absorption of the analytical (characteristic) line, and enhancement of the analytical line owing to excitation by the characteristic line of another element in the sample. Sherman (*122*), who has investigated these deviations theoretically and experimentally for several years, has recently (*8*) treated the difficult enhancement problem with highly encouraging results. In solving the complex mathematical problem of enhancement with monochromatic excitation, Sherman takes into account the various absorption and emission coefficients and derives equations that yield results in satisfactory agreement with observed values. He further indicates how the problem of polychromatic excitation may be solved. Beattie and Brissey (*10*) have given a satisfactory, semiempirical treatment of deviations due to absorption in quaternary high-temperature alloys.

Several other matters of general interest remain. It has been shown experimentally (*94*) that an x-ray emission spectrograph operating under ideal conditions may, as concerns precision, be regarded as a random system in which the predictable standard deviation ($s_C = \sqrt{N}$) is realized. Conversely, if the actual standard deviation exceeds the predictable, operating conditions are not ideal. The latest in a series of investigations concerned with the (small) effect of chemical combination of x-ray spectra is described by Cauchois (*27*). The increasing use of x-ray emission spectrography for the determination of elements in small amounts (*93*) received particular attention at the 1955 Pittsburgh Conference (*8*) and is evident in many of the references cited below. Rhodin (*8*) analyzed thin films, and Pfeiffer and Zemany (*113*) showed that x-ray emission spectrography is adaptable to spot test techniques. Grubb and Zemany (*56*) moved further in the same direction by using an ion-exchange membrane to concentrate traces of ions for subsequent determination in the x-ray spectrograph.

The usefulness of x-ray emission in the petroleum industry is evident (*17, 28, 36, 37, 76, 78, 91, 133*); three different examples will be cited. Lamb, Niebylski, and Kiefer (*78*) extended earlier work of Birks, Brooks, Friedman, and Roe (*17*) to show that x-ray emission spectrography could compete successfully with x-ray absorptiometry in the determination of tetraethyllead in gasoline (*91*) and that variations in the composition of the matrix (everything not being determined—e.g., sulfur, other impurities, and organic halides) were easier to deal with in the emission method. Davis and Van Nordstrand (*36*) used the method for barium, calcium, and zinc in lubricating oils. Determination of the first two elements required the use of a helium path, and certain deviations due to absorption were treated mathematically to give results in accord with experiment. Dyroff and Skiba (*37*) have done much to solve another problem that has recently vexed the petroleum industry—the determination of traces of iron, nickel, and vanadium in cracking catalysts—it being well known that the latter two elements are especially objectionable even when present in minute amounts.

The application of x-ray spectrography to the analysis of minerals generally has progressed rapidly during the past 2 years (*1, 3, 4, 7, 8, 24, 58, 69, 73, 84, 106, 120, 123, 130, 139*). In part, this progress has come because x-ray emission can, with minimum trouble and preferably by use of a helium path, reveal which of the important metals are present in an untreated ore and give a fair indication of their amounts even when these amounts are small. Further impetus derives from the fact that the x-ray method escapes the complexities of wet chemical analysis—e.g., in determinations of elements such as tungsten, tantalum, or the rare earths. Thus, Adler and Axelrod at the U. S. Geological Survey have determined thoria in rock samples with thallium as an internal standard (*2*).

Tournay (*138*), using electron excitation, has found the intensity ratio of an L line of uranium to an L line of thorium to be a simple function of composition, so that the addition of a standard was unnecessary; Tournay (*136, 137*) also applied this method to the determination of niobium and tantalum in cassiterite slags. Campbell and Carl (*23*) at the Eastern Experiment Station of the Bureau of Mines have for several years been investigating x-ray emission techniques for this determination, three of which (establishing niobium-tantalum ratio in separated oxide mixtures; internal standards techniques; use of an additive) gave results believed by them to be more reliable than those by existing chemical methods. These authors (*8*) have carried out a similar study of the determination of germanium in coal and coal ashes.

X-ray emission spectrography continues useful in the determination of major constituents in alloys, as is clear from the papers presented at the Pittsburgh Conferences (*6–8*) and from other references cited in this section. The number of recently published papers dealing exclusively with this application (*19, 61, 62, 101, 102*) is not large, perhaps because the method is proving to be relatively trouble-free. Mihalisin (*102*) reports the saving of time usually realized when x-ray emission spectrography is used in combination with established wet methods in the analysis of high-temperature alloys and cites data showing the x-ray results to be sufficiently reliable for quality control.

Clark, Loranger, Bodnar, and Terford (*32, 33*) combined x-ray emission spectrography with x-ray diffraction and with radiography in a comprehensive analysis of foundry dusts, which sometimes constitute health hazards.

The development of apparatus for x-ray emission spectrography goes forward rapidly. The trends are toward curved crystals to increase intensity and simplify optics (*15, 71*), toward increasing use of the proportional and the scintillation counter (*14, 29, 46, 50, 75, 103, 131, 143*), and of course toward more completely automatic equipment (*66, 70, 72, 116, 129*). The large spectrographs cannot be adequately described here.

Several smaller instruments are of particular interest. Birks and Brooks (*15*) describe a curved-crystal spectrograph for which intensity, resolution, and line-background ratio were as favorable for a 1-mg. sample as for a 10-gram sample in a commercial flat-crystal spectrograph. These authors (*16*) also describe a miniature spectrograph without moving parts that is used on ordinary diffraction equipment with a photographic plate. Castaing and Guinier (*26*) have published an English description of their "electronic microanalyzer," discussed in an earlier review (*91*). Macdonald and Harwood (*99*) have developed equipment unusual in several respects. It combines a spectrograph employing electron excitation with an electron diffraction unit so that an x-ray spectrogram and an electron diffraction pattern could be photographed for the same sample, which gives information at once about ultimate composition and about the compounds present. A plane glass optical diffraction grating was used to disperse characteristic lines ranging from 5 to 200 A. The equipment should prove particularly valuable in the study of surface films. Another vacuum spectrograph, described by Morlet (*104*), provides for velocity selection of the exciting electron beam and is capable of handling *K*- and *L*-spectra in the range 2 to 20 A. Adler and Axelrod (*3*) have designed a "multi-wave-length" spectrograph that provides for the simultaneous measurement of as many as four characteristic lines. Four channels, each containing a detector, a goniometer, an analyzing crystal, and a collimator, converge on a single x-ray tube and sample holder. One of the goniometers doubles as a scanning goniometer. A two-goniometer prototype has been built and applied successfully to the determination of niobium in bauxite ores.

Three valuable techniques related to x-ray emission spectrography employ unusually interesting equipment. Steinhardt and Serfass (*126, 127*) use an "x-ray photoelectron spectrometer" for the study of surfaces. The process studied may be regarded as electron excitation in reverse—here an x-ray beam is used to produce a characteristic electron spectrum in which the variation of photoelectron intensity with photoelectron energy characterizes the sample surface. Hámos (*59*) has built an "x-ray microanalyzer camera," in which a single cylindrically bent crystal is used to reflect onto a photographic plate all the characteristic x-rays excited by an x-ray beam incident upon a very small sample area. The applications tested, which follow, show why this instrument is of great potential value: precise thickness measurements on surface layers between 2×10^{-5} and 3×10^{-4} cm. thick; microanalysis of alloys on volumes near 10^{-8} cc.; concentration variations in surface layers—e.g., determination of microscopic segregations. Finally, though Lindström (*97*) is concerned primarily with absorptiometry (see "Histochemical Analysis" below), his techniques and equipment are of interest to anyone concerned with x-ray emission spectrography at the longer wave lengths.

γ-Ray Spectrometry. This technique (*8, 68, 111*) is restricted to radioactive isotopes and to elements that can be made to yield radioactive isotopes. It is related to x-ray emission spectrography, the principal differences stemming from the fact that nuclei—not atoms—are the sources of γ-rays. As ordinarily practiced, γ-ray spectrometry involves the measurement of the intensity of radiant energy as a function of wave length, and thus goes beyond spectrometry as usually defined—γ-ray spectrography might be a better name. Kahn and Lyon (*68*) have this to say:

> This method simplifies radiochemical analysis, but does not, of course, replace it. The energies of the γ-rays from any one radioisotope are characteristic, but by no means unique; hence, identification by this method may have to be augmented by determination of half life and maximum beta energy, or by chemical separation. While chemical similarity presents no problem, similarity in the gamma energies of two substances in the sample may give misleading results.
>
> Pure beta emitters cannot be identified by this method, but their maximum beta energy may be obtained with the spectrometer.
>
> The gamma spectrometer goes beyond radiochemical analysis in that a particular radioisotope, rather than just an element, is identified.
>
> Applications of the method are numerous. The spectrometer is of use in the solution of many problems in the radiochemical laboratory, in checking procedures, and in analyzing unknown substances.

For reasons given in the section entitled "Detectors," a scintillation counter with pulse-height discrimination is ordinarily the most desirable detector for γ-ray spectrometry.

The identification and study of radioactive isotopes in the atomic energy program and their monitoring in tracer experiments are two important applications of γ-ray spectrometry. In necessarily simple form, the technique is also used in aerial prospecting for radioactive minerals (*112, 119, 128*) and oil (*115*). Peirson and Franklin (*112*) have estimated that uranium can be just detected above background so long as the factor (per cent U_3O_8 times area in square yards) exceeds 100, the observations being made from a plane traveling 120 miles per hour at an altitude of 500 feet. Finally, γ-ray spectrometry is doing much to simplify and to increase the reliability of neutron activation analysis, an example being the determination of impurities in materials of semiconductor grade purity such as silicon (*105*).

HISTOCHEMICAL ANALYSIS

The interesting and important work on x-ray methods of histochemical analysis being carried on by Engström and his collaborators has had only brief mention in earlier reviews (*88, 90–93*). A recent comprehensive publication by Lindström (*97*) emphasizes anew that this research is making a major contribution not only to our knowledge of the living cell but also to the difficult technique of using long-wave-length (2.5 A. and above) x-rays in microanalysis. The two major kinds of analytical problems solved are consequently described here in some detail.

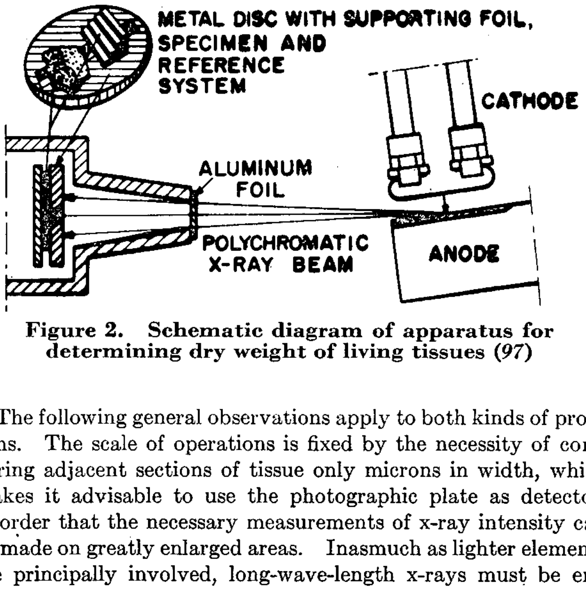

Figure 2. Schematic diagram of apparatus for determining dry weight of living tissues (*97*)

The following general observations apply to both kinds of problems. The scale of operations is fixed by the necessity of comparing adjacent sections of tissue only microns in width, which makes it advisable to use the photographic plate as detector, in order that the necessary measurements of x-ray intensity can be made on greatly enlarged areas. Inasmuch as lighter elements are principally involved, long-wave-length x-rays must be employed. This in turn requires thin samples and a vacuum path. Finally, for work in vacuum, the samples must be dry.

Determination of Dry Weight. The first problem is that of finding the dry weight per unit area, and the variations therein, of various cytologic structures. This problem is solved by means of absorptiometry with a filtered, continuous polychromatic beam of 1.5-kv. peak or 8.3 A. minimum wave length. The analysis is done by the comparative method with a carefully prepared step gage of nitrocellulose foils as standard (see schematic Figure 2).

The reliability of the results depends in large measure on how well deviations from the (ideal) linear relationship between log *I* and dry weight per unit area can be eliminated or allowed for. It is well known, this can be accomplished by the comparative method (*144*), provided that standard (reference system) and unknown, identical in mass, shape, and elementary composition, are exposed to the same x-ray beam. In the cytological investigations, these conditions are difficult to meet, not only because the samples are complex in composition, but also because they are very small, as is clear from the units employed (micromicrograms per square micron or 10^{-12} gram per 10^{-8} sq. cm.).

Fortunately, some simplifications are possible. Carbon, nitrogen, and oxygen in tissue proteins are the elements principally responsible for the absorption of x-rays by the different biological structures. Furthermore, the elementary composition of proteins varies little even though they are made up of various amino acids in greatly different proportions. Nitrocellulose foils therefore make satisfactory standard step gages. The elementary compositions of these standards and of the unknowns are similar enough so that differences in x-ray absorption can be allowed for on the basis of the systematic "errors" (some positive, some negative) that can be approximated in advance. Lindström (*97*) has computed such systematic errors not only for the important constituents of biological materials at six wave lengths in the range 2.5 to 22 A., but also for the polychromatic beam actually employed. (Only polychromatic beams can meet the high intensity requirements imposed by the low sensitivities of the fine-grained emulsions employed to ensure sufficiently high resolution.)

Table VI. Data on 20 Cells

Cell No.	u_c	a, Microns	m_c, Micro-micrograms
1	0.97	17.9	377
2	0.74	18.1	293
3	0.97	18.8	416
4	0.925	17.8	355
5	1.45	17.7	551
6	1.10	15.8	333
7	0.95	16.3	306
8	1.54	15.7	461
9	1.49	17.0	522
10	1.22	16.1	384
11	1.015	19.0	444
12	1.67	17.3	606
13	0.85	19.1	376
14	1.10	16.8	377
15	0.95	16.9	329
16	0.995	16.3	321
17	1.10	17.3	400
18	0.85	18.0	334
19	1.22	17.5	454
20	0.70	17.7	266

An impressive demonstration of what can be done with the method is provided by Figure 3, which shows a microradiogram of a section of striated muscle, an excellent test object.

In the figure, anisotropic bands (white, 0.5 to 0.7 micron wide) of high dry-weight per unit area alternate with broader (dark, 1.3 to 1.5 microns wide) isotropic bands whose dry weight per unit area is lower in the ratio 1 to 1.41 ± 0.05. The ratio is the mean of ten values obtained by photometering areas of about 1.5 square microns (1.5×10^{-8} sq. cm.) each, and the small uncertainty in the mean includes the natural variations in the tissue!

The kind of detailed quantitative information the method can yield is well illustrated by the determination of the total dry weight of individual ascites tumor cells, the volume of each cell being less than 4×10^{-9} cc. The total dry weight, m_c, of a cell is given by

$$m_c = 1.32\, u_c w \pi a^2/6 \text{ (for units, see Table VI)}$$

where

u_c = number of standard foils equivalent in absorbance to the sample

w = weight per unit area (1.775 micromicrograms per sq. micron) of one standard foil

a = diameter of cell

The data for twenty individual cells are given in Table VI.

The mean value of the dry weight, $(3.95 \pm 0.20) \times 10^{-10}$ gram, agrees with results obtained in microchemical analyses of samples containing many cells. Such analyses cannot, of course, reveal the individual variations that appear in Table VI.

Elementary Analyses on a Cytochemical Scale. Calcium, phosphorus, and sulfur have been quantitatively determined in biological samples comparable in volume with the living cell (*97*). The initial step in this direction was taken by Engström (*41*), who was the first to show that the method of Glocker and Frohnmayer (*53*), could be successfully applied to microtome sections of biological materials. At the long wave lengths necessary, the problem of obtaining high enough intensity in the monochromatic beam is especially acute. This makes it desirable to modify the original method of Glocker and Frohnmayer whenever possible, by using characteristic lines of suitable elements to replace the usually less intense monochromatic x-rays diffracted by a crystal from a polychromatic beam. This modification, made by Moxnes (*109, 110*) and by Engström (*42*), will not be given a special name.

To carry out the analyses, it was necessary to design and build a high-vacuum x-ray spectrophotometer that employed a curved crystal (usually quartz with an appropriate radius of curvature) to ensure sufficient intensity. The photographic plate served as detector, and exposure times ranged from 30 minutes to 4 hours. Only one wave length near each side of the absorption edge was used, and the distance of each wave length from the edge was appropriately allowed for. For the determination of calcium (absorption edge near 3.1 A.) the monochromatic beams had to be taken from a polychromatic beam of 7 kv., because suitable characteristic lines were unavailable. In the case of phosphorus (absorption edge near 5.8 A.), the $L\alpha_1$ line of niobium and the $L\beta_1$ line of zirconium were used. The $L\alpha_1$ line of ruthenium and the $L\beta_1$ line of molybdenum served for determining sulfur (absorption edge near 5.0 A.).

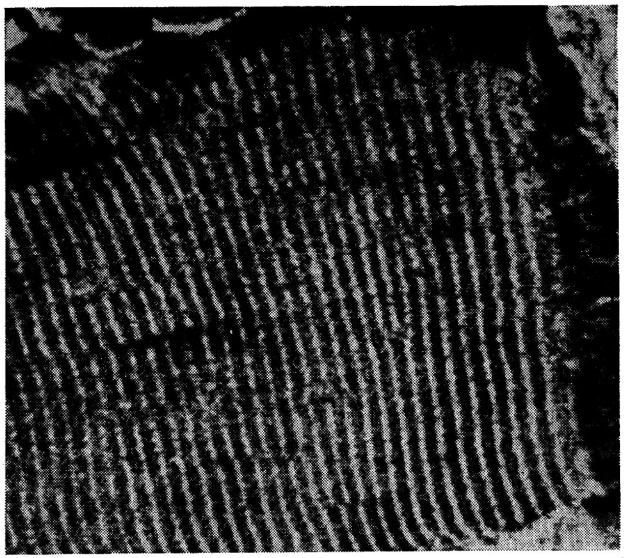

Figure 3. Microradiogram of 3-micron thick section of formalin-fixed striated muscle of *Chironomus*

1.0-kv. x-ray beam (*97*) (× 1200)

The accuracy and precision of the results may be judged from the following. In sections of biological materials, about 10 microns thick, the absolute errors in the determination of calcium ranged between ±0.15 and ±0.2 micromicrograms per square micron; those for phosphorus and sulfur between ±0.06 and ±0.1 micromicrograms per square micron. These errors often corresponded to less than 5% of the amount of element present in the sample.

Although the accuracy and precision of the method are remarkable in light of the minute samples, the outstanding feature

Table VII. Research Results for Biological Materials

Sample	Conclusions[a]
Human bone tissue	Single Haversian systems differ significantly in calcium-phosphorus ratio.
Arterial wall in arteriosclerosis	Dense, homogeneous, calcified section of wall shows 3.3 times as much calcium as phosphorus.
Human kidney section	Homogeneous section of renal calculus contains 5.3 ± 0.2 micromicrograms calcium per square micron.
Sea urchin egg	Nucleolus contains about 4.4% phosphorus.
Rat epidermis	Corneal and spinous layers are roughly identical in sulfur concentration.

[a] Based on analytical results by new methods on necessarily restricted number of samples, may subsequently need to be modified.

of the work is this minuteness itself, which makes it possible to discover how the chemical composition of biological materials varies from one small section to another. The data thus obtained can be combined with dry-weight data from absorptiometry with polychromatic beams to yield conclusions of the kind listed in Table VII.

Owing to the complexity of living tissue, the x-ray techniques described above will often need to be supplemented with others. Recent work shows the progress that can be expected to follow from such a concerted analytical approach (*11, 25, 38–40, 43, 98*).

ACKNOWLEDGMENT

For help in the preparation of this review, the authors wish to thank Bo Lindström, Karolinska Institutet, Stockholm, William Parrish, Philips Laboratories, and several of their colleagues, notably H. G. Pfeiffer and P. D. Zemany.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

X-Ray Diffraction

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IN THE present review no effort has been made to comment on, or even to list, all papers dealing with x-ray diffraction which have appeared during the past year. Rather, an effort has been made to limit the discussion to items which are believed to be of general interest to chemists; emphasis has been placed on analytical methods and crystallographic studies of organic and inorganic compounds.

Several books dealing with various aspects of x-ray crystallography have appeared during the past year; two are especially noteworthy. Both deal with "powder" methods of x-ray diffraction—i.e., the x-ray studies of polycrystalline materials. "X-Ray Diffraction Procedures" by Klug and Alexander (*44*) is an excellent manual of techniques; "X-Ray Diffraction by Polycrystalline Materials" by Peiser, Rooksby, and Wilson (*59*) is a collection of essays which deals extensively with a wide variety of topics in x-ray diffraction. Both volumes present large amounts of material not readily available without a thorough search of the literature.

Two journals are now devoted exclusively to publication of crystallographic papers: *Acta Crystallographica* and *Zeitschrift für Kristallographie.* The latter resumed publication in October 1954, after discontinuing publication during the war.

APPARATUS AND TECHNIQUES

A goniometer has been described which is designed to facilitate the investigation of diffraction intensities from single crystals using Geiger counters (*30*). The crystal may be rotated about each of the Eulerian axes; each reciprocal lattice point may be brought into reflecting position in the equatorial plane where all measurements are made.

A careful comparison has been made of Geiger counter and photographic techniques in single crystal x-ray studies (*78*). The Geiger counter method is found to be somewhat more reliable than the photographic, but is more time-consuming. The usefulness of the improved accuracy is being tested in various crystal structure determinations now in progress.

A careful study has been made of the available x-ray diffraction detectors (*71*). These include the conventional end-window Geiger counter tubes as well as more recently developed side-window proportional counters and scintillation counters. Methods have also been described for x-ray diffraction studies of radioactive materials using diffractometric techniques (*46*). Usually, thick layers of lead are placed strategically to cut down unwanted background; the authors show how scintillation counters or proportional counters, with pulse height discrimination, may be used to cut background to very small values without using lead shields.

A device for computing two-dimensional Fourier series by optical methods has been described (*28*). The instrument appears to be superior, for this purpose, to the well-known "Huggins masks" which were designed for the same purpose. Examples are given of the use of the newer device in the determination of the crystal structure of *p*-toluidine hydrochloride; it has also been found useful in the computation of "difference" Fourier series.

Interest in the use of high-speed digital computers for crystal structure calculations continues. Robertson (*64*) has described a fast digital computer for Fourier operations which is the mechanical analog of the widely used Beever Lipson strips. Cochran (*18*) has discussed the possible utilization of high-speed computers for the direct determination of crystal structures. He describes a method whereby the computer can be used to select a set of signs for the coefficients of the Fourier series such that the Fourier series satisfies given conditions. Examples are given of the successful application of the method to the determination of the structures of some crystals of moderate complexity and of its unsuccessful application to the structures of somewhat more complex crystals (nitroguanidine and asparagine).

Brown, Moneypenny, and Wakelin (*15*) have discussed procedures for measurement of intensities of diffraction lines on film photographs of powdered specimens. They describe a servo-controlled microdensitometer for automatic scanning of film photographs which is accurate to 0.004 part in three density units.

The precision attainable in the determination of lattice constants using the Geiger counter diffractometer has been discussed in detail (*69*). Precise lattice constants have been determined for a number of elements and simple cubic compounds. It was found that the limiting factor in the accuracy attainable by this method was the inaccuracy in the knowledge of the wave length of the x-radiation used. The lattice constants could be measured with an accuracy of one part in 250,000, but our knowledge of the absolute values of x-ray wave lengths is only half as accurate.

A theoretical investigation of the thermal motion of atoms in molecular crystals by Higgs (*39*) indicates that there exists a linear relation between the root mean square amplitude of atomic motion and the square of the distance of the atom from the center of mass of the molecule. It is felt that this type of thermal libration may account in large part for the variations in the electron density of carbon peaks which have been reported for anthracene and naphthalene.

Improved precision of crystal structure determinations in recent years has made possible the location of hydrogen atoms in the electron density maps of many molecules. McConnell (*49*) has compared optical methods of testing possible H-atom positions with the more objective difference synthesis. The latter is found to be more definite, but the former is more useful in noncentrosymmetric projections where difference methods cannot be used.

IDENTIFICATION OF CRYSTALLINE SUBSTANCES

The use of x-ray diffraction data for the identification of crystalline substances is continuing its rapid growth. With few exceptions this technique utilizes "powdered" specimens; it is nondestructive and may be used effectively with milligram, or even microgram, quantities. During the past year emphasis has been placed particularly on the application of the method to the identification of organic compounds.

A group of Canadian workers has been particularly active in this connection. Barnes, Donaldson, and Phillips (*6*) have published patterns of various alkaloids for identification purposes. Barnes and others have also published large numbers of patterns of narcotics (*7–9*).

X-ray powder patterns of 52 aliphatic and aromatic amine hydrochlorides (*14, 45*), of many tetrazole derivatives, and of large numbers of steroids (*57, 58*) have been published recently in ANALYTICAL CHEMISTRY.

A section devoted to crystallographic data is also a regular monthly feature of this journal.

Rose (*65, 66*) has published patterns of alkaloids and other compounds of pharmaceutical interest.

The National Bureau of Standards is continuing the publication of carefully collected standard powder diffraction patterns of inorganic compounds and elements. During 1955, two volumes devoted to these x-ray patterns have been published (*55*). All the patterns in these volumes, together with hundreds of others, including the organic patterns mentioned above, are included in the newest set (6th) of the ASTM card file of powder diffraction patterns which has just been published.

ALLOYS AND ELEMENTS

In solid solutions random displacements of both the solvent and solute atoms from their mean positions occur as a result of the elastic strains associated with the different effective sizes of the two types of atoms in the crystal. A careful study of the integrated intensities of reflections from 15% solutions of gold in copper has enabled Coyle and Gale (*20*) to determine the root mean square atomic displacement due to distortion. In this calculation it was first necessary to allow for displacements due to thermal vibrations, which affect the x-ray intensities in a manner similar to the distortion being investigated. It was found that the root mean square "distortion" displacement was 0.11 A., compared with a value of 0.13 A. computed from elasticity theory.

Heaton and Gingrich (*38*) have re-examined the structure of Mn_2Sb. Atomic parameters were refined and these new values were used in a careful investigation of the integrated intensities of selected reflections over the temperature range from 100° to 500° K. From the variation of these intensities with temperature it was possible to compute Debye characteristic temperatures; for reflections from the (00*l*) planes it is 300° K. and for (*h*00) reflections it is 280° K. Evidence of changes in the Debye temperatures at elevated temperatures was reported.

A careful investigation has been made of the lattice expansion of iron from 20° to 1502° C., using high temperature x-ray diffraction techniques (*5*). In combination with earlier low temperature work, the new data yield complete experimental information regarding the thermal expansion of iron from 0° K. to the melting point (1534° C.).

A study of a rhombohedral modification of natural graphite has been carried out by Boehm and Hofmann (*13*). The rhombohedral modification is fairly common in selected specimens of natural graphite, but only one specimen of artificial graphite revealed the presence of this form, and then only in small amounts.

Single crystals of graphite were invariably found to be of the usual hexagonal type. On heating to 2000° to 3000° C. the rhombohedral form is converted to the hexagonal type, which is considered to be the stable modification for all temperatures below 3000° C.

INORGANIC COMPOUNDS

Efforts to determine the exact electron distribution in simple crystals have been described by Wagner, Witte, and Wulfel (*75*). Details of the experimental setup for accurate intensity measurements are given. In the sodium chloride crystal slight deviations from spherical symmetry of the charge distribution of the two ions were detected. The calculation indicated that 10.05 electrons were associated with the sodium ion and 17.70 with chlorine—i.e., ionization is essentially complete.

Thewlis (*72*) has made precise measurements of the lattice dimensions of Li^6F and Li^7F; the cubic unit cells were found to have lattice constants of 4.0271 and 4.0263 A., respectively. The difference, 2 parts in 10,000, is well beyond the limits of experimental error and is attributed by the author to differences in zero point energy between the two lithium isotopes. A difference of about 3.3 parts in 10,000 between the unit cell dimensions of the two types of lithium fluoride has been predicted theoretically.

The effect of nuclear radiation on the structure of zirconium silicate has been investigated (*40*). It was found that during the course of the irradiation the density of the zircon dropped by 16%; it became isotropic and so disordered that it failed even to give recognizable x-ray diffraction peaks. The observed effects are attributed to the displacement of atoms by recoil nuclei and by the high temperatures generated in the irradiation process. The mechanism of the structural breakdown is discussed.

Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) undergoes a phase transition at 140° K. Pepinsky and Keeling (*43*) have examined the structure of this compound at 5° below and 5° above the temperature of the transition. They found that on passing through the transition the low temperature orthorhombic unit cell changes to one of tetragonal symmetry. It is shown that charge displacements take place in the transition which lead to antiferroelectricity below the transition temperature.

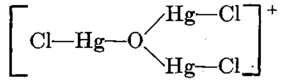
Little was known until recently concerning the stereochemistry and bond lengths of cyclic siloxane compounds. Peyronel (*61*) has found that the six-membered (Si—O—Si—O—Si—O) ring of hexamethyl cyclotrisiloxane is planar within the limits of experimental error; the Si—O bond length is 1.61 A., Si—C is 1.93 A.; the O—Si—O angle is 136°.

The crystal structure of the analogous eight-membered ring compound (octamethyl cyclotetrasiloxane) has been determined at −45° C. (*70*). The siloxane ring is puckered; bond lengths are Si—O, 1.65 A.; and Si—C, 1.92 A. The Si—O—Si angle is 142.5° and the O—Si—O angle is 109°. A solid phase transformation occurs at −17° C.; above that temperature—i.e., between −17° and the melting point, 16° C.—individual molecules may assume randomly any one of four alternative orientations. The onset of disorder is marked by an increase in crystal symmetry above the transition temperature.

Kasai and Kakudo have computed two-dimensional electron density maps of diallylsilanediol (*42*). They found that the molecules are linked in the crystals in the form of infinite O—H—O bonded chains; the O—H—O bonds are 2.53 A. long; the Si—O bond is 1.63 A., and the Si—C bond is 1.90 A., in good agreement with measures of the same bonds in the cyclic siloxanes. The O—Si—O and the C—Si—C angles were reported as both 110° within experimental error.

A study of the crystal structure of alpha-KO_2 (*1*) has revealed two types of anion-cation closest contacts in the crystal: one of 2.71 A., the other 2.92 A.. Similar results had previously been found for BaO_2 by the same authors—i.e., 2.68 and 2.79 A. The O—O bond is 1.28 A. long; its order is estimated to be close to 0.5.

A somewhat surprising molecular arrangement has been found in crystalline BrCN (*33*). Molecules of BrCN are arranged in the solid in the form of straight chains with the bromine atom of one molecule weakly linked to the nitrogen atom of the next molecule in the chain.

The crystal structure of trichloromercury oxonium chloride has been determined (*67*). The structural units were found to consist of $(ClHg)_3O$ cations and Cl anions. The conventional way of writing the formula of this compound—i.e., $2HgCl_2.HgO$—is therefore incorrect. The  ion is planar, with oxygen, mercury, and chlorine atoms very nearly in a straight line.

The crystal structure of SnI_4, originally determined by Dickinson in 1922, has been reinvestigated (*53*). It was found that Dickinson's structure is essentially correct but that the observed diffraction pattern can be adequately explained without invoking all deviations from the "ideal" structure used by Dickinson.

The structure of $TiOF_2$ has been determined from x-ray powder photographs (*73*). Titanium atoms are octahedrally coordinated by randomly distributed oxygen and fluorine atoms. The octahedra share all six corners with neighboring octahedra. The authors feel that powder diffraction data, previously attributed to TiF_4, are probably due to $TiOF_2$.

The crystal structure of AlN was originally determined in 1929; it was then assigned the ideal wurtzite structure. Jeffrey and Parry (*41*) have reinvestigated the structure and find two types of deviations from the ideal wurtzite structure: the axial ratio of the hexagonal cell is 1.600 instead of 1.633, and the parameter which determines the Al—N bond length is 0.385 instead of 0.375. Two types of N—Al—N angles, 107.7° and 110.5°, are present; Al—N spacings are 1.885 and 1.917 A.

Hamilton has completed a detailed study of the crystal structure of the dimethyl phosphinoborine trimer (*37*). The determination was based on the use of full data in three dimensions; results were refined by least square and difference Fourier techniques. The structure consists of cyclohexane types of rings of alternating phosphorus and boron atoms; two methyl groups are bonded to each phosphorus atom and two hydrogen atoms are bonded to each boron atom.

Vos and Wiebenga have determined the crystal structure of P_4S_{10} and P_4S_7 (*74*). In the former they found S—P—S and P—S—P angles of 109.5°. In P_4S_7 some of the S—P—S angles deviated significantly from 109.5°. In both structures two types of P—S bonds may be distinguished with bond lengths of 2.08 and 1.95 A. The structures are made up of discrete molecules of P_4S_{10} and P_4S_7, respectively.

An interesting structure has been proposed by Edstrand and Blomquist for $NH_4Cl.As_2O_3.{}^1/_2H_2O$ (*25*). The atoms appear to be arranged in sheets perpendicular to the *c* axis of the hexagonal unit cell. The structure contains uncharged layers of arsenic trioxide with all arsenic atoms arranged in pairs on one side of the oxygen sheet. Water molecules are probably located in holes in the layers. Between layers of arsenic trioxide are layers of ammonium chloride molecules.

An investigation of the crystal structure of calcium monochloride, CaCl, has been completed by Ehrlich and Gentsch (*26*). The substance crystallizes in the form of a layer structure; double layers of Ca—Ca alternate with Cl—Cl double layers. All layers are parallel to one another. The shortest Ca—Ca distance is 3.38 A., twice the calculated Ca^{+1} radius. In the Cl layer, the bonding appears to be of the van der Waals type.

Geller and coworkers have continued their crystallographic investigation of the phases formed in various binary systems. The crystal structure of CO_2Si was redetermined and found to be a distorted Ni_2In structure (*34*). The structure of $RhSe_2$ was determined from powder data (*32*). In the Rh—Ge system, the crystal structures of the Rh_2Ge, Rh_5Ge_3, and PhGe phases, all previously unreported, have been determined (*31*).

A low temperature study of the crystal structure of HNCO has been completed (*23*). Below about −100° C., two phases appear to be stable; the structure determined was that of the form stable from −100° to the melting point. Molecular dimensions are similar to those in the vapor phase. Surprisingly, no N—H—O bonds were found; the molecules are held together by N—H—N bonds, about 3.07 A. in length.

A low temperature (−60° C.) study has been made of the crystal structure of hydroxylamine (*54*). The N—O bond is 1.476 A., in good agreement with other values determined for single N—O bonds. Two types of N—H—O bonds were found: one approximately 2.74 A. long, the other about 3.10 A. long. The third hydrogen atom in each molecule does not appear to participate in hydrogen bonding.

A cagelike (clathrate) structure has been found in crystals of $HPF_6.6H_2O$ (*12*). The water molecules form cubo-octahedral cages of twenty-four oxygen atoms about each PF_6 group. In the center of each cage is a phosphorus atom.

ORGANIC COMPOUNDS

A recent redetermination of the crystal structure of benzene has been refined further by Cox, Cruickshank, and Smith (*19*). Cox had reported surprisingly short C—C bond lengths of 1.378 A. in 1954. In the present work, a careful re-examination of the data, taking into account the anisotropy of thermal motion of the carbon atoms—i.e., movements perpendicular to the plane of the ring as well as radially and tangentially within the plane of the ring—indicated that the C—C distance is 1.39 A., in better agreement with values determined by other methods.

Work is continuing on the crystal structure of 9,10-dihydroanthracene (*29*). Although some remarkable similarities have been observed between the structure factors of this compound and those of anthracene, 9,10-dihydroanthracene, unlike anthracene, is not planar. The molecule is bent at an angle of about 145° along a line, joining the two central carbon atoms.

The crystal structure of fluorene (*17*) has been determined; its carbon skeleton is planar to within experimental error. Bond lengths are discussed in detail in the paper.

A precise determination of the structure of *s*-triazine using full data in three dimensions has been carried out by Wheatley (*77*). The molecule is planar, but not a regular hexagon; the C—N distance is 1.319 ± 0.005 A.; the N—C—N angle is 126.8°, compared with a C—N—C angle of 113.2°. A plot of C—N bond length *vs.* bond order indicates that the 1.319 A. C—N bond distance corresponds to approximately 50% double bond character.

Marsh has completed a determination of the crystal structure of 1,4-dithiane (*52*). Precise values of atomic parameters were obtained by a least squares refinement of full three-dimensional data. Like 1,4-diselenane, the 1,4-dithiane molecule has the centrosymmetrical chair configuration. Unlike 1,4-diselenane, in which the Se—C bond is appreciably longer than the sum of the covalent radii of selenium and carbon the S—C bonds in in 1,4-dithiane (1.80 and 1.821) are "normal"; the sum of the covalent sulfur and carbon radii is 1.81 A.

A determination has been made of the crystal structure of benzene seleninic acid (*50*). The molecules are found to be linked together in infinite chains by O—H—O bonds only 2.52 A. long. Selenium to oxygen bond lengths of 1.707 and 1.765 were reported; other bonds were normal.

Benzoic acid has been found (*68*) to crystallize in the form of centrosymmetric dimers; the dimers are almost planar and are held together by hydrogen bonds 2.64 A. long between carboxylic oxygen atoms. The two carbon to oxygen bonds in one carboxylic group were reported as 1.29 and 1.24 A. long.

Electron density difference maps computed for *p*-aminosalicylic acid show peaks which have been attributed to hydrogen atoms (*10*); two-dimensional data were used. Inter- and intramolecular hydrogen bonding between oxygen atoms are found in the crystal, as well as indications of weak O—N and N—N hydrogen bonds.

The crystal structure of creatinine consists of strings of molecules linked by hydrogen bonds (*24*). The molecule is essentially planar; intramolecular bond lengths indicate a nearly complete system of resonance with the exception of one N—C bond.

Gupta (*36*) has continued work on the structure of $C_4H_4O_6.2H_2O$; previously (*35*) he had established that the configuration is trans—i.e., that the compound is dihydroxyfumaric acid. In the more recent work he has confirmed the trans configuration by means of electron density maps. No hydrogen bonding between COOH groups of adjacent molecules was observed; the structure is held together by molecules of water.

A precise determination of the crystal structure of parabanic acid (*22*) has been carried out by Davies and Blum. Three-dimensional data were used in the determination; a least squares refinement of the structure was carried out, followed by the computation of three-dimensional difference maps of electron density. The latter revealed the location of peaks with an electron density of approximately 0.7 electron per $A.^3$, 1 A. from each of the nitrogen atoms. These were evidently hydrogen atom peaks. Use of all the available diffraction data together with the extremely careful refinement process reduced the average standard deviations of atomic locations to ±0.003 A. and those of bond lengths to ±0.004 A. The C—C bond is 1.541 A. long, in good agreement with previously determined C—C single bond lengths; the carbon to oxygen bonds range from 1.208 to 1.212 A. and are somewhat shorter than expected. The C_1N_2 and C_1N_1 bonds are 1.380 and 1.382 A., and the N_2C_3 and N_1C_3 bonds are 1.360 A. long. All appear to possess a high degree of double bond character.

Nowacki and Burki (*56*) have determined the structure of xanthazol monohydrate by two independent methods: one was based on parameters computed from a three-dimensional Patterson analysis; the other was based on signs of Fourier coefficients determined by the use of Harker-Kaspar and Zachariasen inequalities. There are two, almost coplanar molecules, per unit cell; they are held together by hydrogen bonds; the hydrogen atoms in the bonds show up clearly in the electron density maps.

Eiland and Pepinsky (*27*) have described the crystal structure of cyclotetramethylene tetranitramine. Full data in three dimensions were used in the investigation. The nitramine group is planar; N—N bonds are 1.38 and 1.40 A. long. Intermolecular C—O bonds of 3.01 and 3.12 A. were found.

Blackmore and Abrahams have determined the structures of the isomorphous di-*p*-tolyl telluride, selenide, and sulfide compounds (*11*). Bond lengths and intermolecular distances were of the expected magnitudes; the normals to the two aromatic rings were inclined at the angle of 62° to one another in the case of the telluride, 55° for the selenide, and 56° for the sulfide.

An interesting example of the "sandwich" type of metallo-organic compound, dicyclopentadienyl manganese, has been investigated by Weiss and Fischer (*76*). The structure resembles that of a double cone with a manganese atom at the center and with the C_5H_5 groups serving as the basal planes of the two cones.

LARGE MOLECULES AND HIGH POLYMERS

In determining the crystal structure of codeine hydrobromide dihydrate, Lindsey and Barnes (*47*) obtained the signs of phases of reflections, in three zones, by direct comparison of intensities with those of codeine hydroiodide dihydrate. The structure was deduced from electron density projections onto (*h*00), (00*l*), and (0*k*0). The stereochemical configuration assumed for codeine by organic chemists was fully confirmed; although the bond lengths and angles found by Lindsey and Barnes differed in some instances from those usually assumed, it was not possible to establish whether these differences were statistically significant.

The crystal structure of morphine hydroiodide dihydrate has been determined (*51*). Analysis of electron density projections made possible the location of all the atoms (except for hydrogen) in the unit cell. The spatial configuration of the molecule has been unequivocally established; the structure generally assumed by chemists is correct.

Bunn and deP. Danberry (*16*) have determined the crystal structure of polyethylene terephthalate (Terylene) from the x-ray diagrams given by drawn fibers. The positions of atoms in the crystal were found by the usual Fourier methods. The molecules are nearly planar; bond lengths and van der Waals distances are "normal." From comparisons of the density of crystals of polyethylene terephthalate (1.455 grams per cc.) with that of amorphous material (1.335 grams per cc.) it was possible to establish the proportion of crystalline material in ordinary drawn yarn (48%).

Aggarwal and Tilley (*3*) have described methods of determining the crystallinity of polyethylene by x-ray diffraction methods using a Geiger-counter diffractometer. Alternative procedures for determination of per cent crystallinity in oriented and unoriented specimens are discussed.

It is well known that small angle x-ray scattering yields useful information concerning the size and shape of protein molecules in solution. Beeman and coworkers (*4*) have carried out a detailed analysis of small-angle x-ray scattering from solutions of bovine serum albumin, human mercaptalbumin, and mercaptalbumin-mercury dimer over a wide range of experimental conditions. Radii of gyration of 29.8 A. for bovine serum albumin, 31.0 A. for human mercaptalbumin and 37.2 A. for the human mercaptalbumin dimer are obtained. Molecular models reasonably consistent with both the small angle scattering and single crystal work have been computed.

Liquori has studied the molecular configuration of stretched polyisobutylene (*48*). The configuration is helical, with $^8/_5$ monomer per turn. The C—C—C angle within a chain is 114°.

Three-polymeric forms of crystalline reduced human hemoglobin have been found (*60*); all appear to belong to the same space group. The analysis of the Patterson projection of the simplest of the three indicates that the two molecules in the unit cell are almost parallel to one another.

Ramachandran and Kartha have proposed (*62*) a coiled-coil structure for collagen; three polypeptide chains, each with a threefold screw axis, are assumed to wind slowly about one another to yield a coiled coil. Rich and Crick (*63*) agree with the coiled-coil aspect of the structure but criticize the proposed arrangement of polypeptide chains.

The crystal structure analysis of vitamin B-12 (*21*) has established the molecular configuration of this compound. Full details of the analysis have not yet been published; the determination is a landmark in crystal structure analysis, as this is the largest molecule yet to be attacked successfully by the methods of x-ray diffraction.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Raman Spectroscopy

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THIS review covers papers published since the preparation of the last review in this series (*28*). In a few cases earlier papers, not previously reviewed, are cited. Those papers are not included which present Raman spectra solely for theoretical molecular determination by means of frequency assignments to normal vibrations. The period covered is notable mainly for developments in instrumentation.

APPARATUS

White, Alpert, and DeBell (*31*) describe a grating photoelectric spectrometer and auxiliary apparatus such as an image slicer, multiple traversal Raman tube, and internally water-cooled spiral mercury arc. The Perkin-Elmer Corp. (*17*) has announced the commercial availability of a grating instrument based on a standard monochromator. Another interesting instrument was placed on the market by the Applied Physics Corp. (*8*). This makes use of a double grating monochromator to minimize the undesirable effects of stray radiation and Tyndall scattering from the sample. To achieve maximum utilization of light the monochromator has two entrance slits, an ingenious image slicer is used, and the detecting system includes a rotating sector mirror and two photomultiplier tubes which receive light alternately. Excellent spectra are obtained with a sample volume of 0.1 ml. and a scanning speed of 10 cm.$^{-1}$ per second.

Skinner (*27*) discusses the interrelationship of slit width and scanning speed. Brandmüller (*6*) studies the illumination of a spectrograph by light from a Raman tube. He points out some advantages in not using a condenser lens between the tube and the spectrograph slit. Brandmüller and Moser (*7*) give details for the conversion of a Steinheil spectrograph to a recording spectrometer. Welsh and coworkers (*30*) describe the apparatus they use in their outstanding work on the Raman spectra of gases. A high-current, water-cooled mercury arc lamp is reported by Shull (*26*).

INTENSITIES

There is considerable activity in the study of the theoretical and experimental factors which determine the observed intensities of Raman lines. This work offers the interesting possibility that Raman line intensity relative to some standard line (such as the 459 cm.$^{-1}$ line of carbon tetrachloride) may be obtained independent of the apparatus used. If this can be done, then calibrations for quantitative analysis obtained with one apparatus may be transferred to another apparatus without significant loss of accuracy.

Bernstein and Allen (*5*) have carried out a careful study of the problem of expressing the integrated intensities of Raman lines in terms of a standard intensity scale. They include all corrections which appear to be relevant. Luther and Lohrengel (*15*) express Raman line intensity in terms of a molar scattering coefficient. On the basis of an examination of a number of analytically useful lines of normal paraffins, they conclude that the molar scattering for C-H frequencies can be calculated from group increments. An experimental study of the intensities of the lines of CCl_4, $CHCl_3$, and CH_2Cl_2 is reported by Marrinan and Sheppard (*16*). Rosenbaum, Cerato, and Lauer (*24*) suggest the use of a tungsten lamp standardized as Illuminant A by the National Bureau of Standards to correct an observed spectrum for the wave-length dependence of photomultiplier tube sensitivity and transmission of the spectrometer. Shorygin, Kuzina, and Osityanskaya (*25*) discuss the effects of changes in molecular structure on the intensities of characteristic lines.

ANALYTICAL APPLICATIONS

Robert (*22*) describes the application of Raman spectra obtained with a recording spectrometer converted from a spectrograph to the analysis of petroleum products such as gasoline cuts, aromatic concentrates, and "white" oils (using Hg 5461A excitation). He also presents data for acetone and isopropyl alcohol mixtures in aqueous solution. Xylene isomers are determined by Takei (*29*). The products of low molecular weight in the aluminum chloride–catalyzed polymerization of ethylene to lubricating oils are analyzed by Geiseler, Kaufhold, and Runge (*10*). The effect of a variety of substituents on the ring frequencies of substituted cyclohexanes has been studied by Chiuroglu, Doehaerd, and Maquestiau (*9*). Piaux and Gaudemar (*19*) have determined the purity of allenic hydrocarbons obtained by the propargylic rearrangement.

Variations in the frequency shift of the carboxyl line in branched aliphatic ketones are reported by Heilmann and Arnaud (*12*) and similar considerations for $C=C$ and $C=O$ lines are presented by Kirrmann, Federlin, and Bieber (*14*). Batuev and Antsus (*2*) identify an oxidation product of diisobutylene. Artamonov (*1*) has investigated the acids formed in the hydrogenation of vegetable oils. The three-component mixture of mono-, di-, and trichloroacetic acids in the molten state has been analyzed by del Pilar Jorge and Barcelo Matutano (*13*). Goubeau and coworkers (*11*) use the combination of Raman and infrared spectroscopy to identify the trimer of hydrocyanic acid as 1,3,5-triazine. The products of the reactions between sulfuric acid and phenol and sulfuric acid and cresol are determined by Pershina and Raskin (*18*).

Baudler has investigated the spectra of esters of phosphoric and phosphorus acids (*4*) and aqueous solutions of hypophosphorus acid (*3*). He presents characteristic frequencies for a number of bonds. The complex cadmium and mercuric halide anions in aqueous solutions have been studied by Rolfe, Sheppard, and Woodward (*23*). Woodward and Bill (*32*) use the intensities of lines attributed to $InBr_4^-$ to follow the extraction of indium bromide by ethyl ether and methyl isobutyl ketone from aqueous solutions containing hydrobromic acid. The structures and stabilities of complexes between diborane and methyl ether, ethyl ether, or tetrahydrofuran are discussed by Rice and Uchida (*21*). Shifts in the position of the $C=O$ line in salicylaldehyde are used by Puranik (*20*) to study the transition from intramolecular hydrogen bonding to intermolecular hydrogen bonding.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Electron Microscopy

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TRENDS reported in the previous review (*235*) have continued to develop in a manner that has given electron microscopy a responsible role in delineating the relationship of the microstructure of solids with their properties and behavior. This method in conjunction with other methods of analysis has not only established itself as a necessary line of research in chemistry, physics, and biology but is becoming a valuable aid in industry, agriculture, and medicine.

Engineering developments in the transmission magnetic-type electron microscope have, in the past decade, made the instrument into a reliable laboratory tool. With most major countries manufacturing at least one electron microscope, recent advances in electron optics should continue to be incorporated in the newer commercial designs. Most current models are capable of a resolution of 20 A. (Radio Corp. of America, Camden, N.J., Type EMU-3B; North American Phillips Co., Inc., Mt. Vernon, N.Y., Type EM-100A); one is capable of 6 A. (Siemens & Halske Aktiengesellschaft, Wernerwerk für Messtechnik, Karlsruhe, Germany, Elmiskop I). Present knowledge about the interaction of electrons with matter indicates that a practical limit to the resolving power of the electron microscope is about 5 A. Although this figure is two orders of magnitude worse than the theoretical limit imposed by the wave nature of electron beams, there is hope that the electron microscope may yet make visible the ultimate goal—the structure of the atomic lattice.

Considerable effort still centers around problems related to optimum performance of the instrument. Approximately 1000 electron microscopes are in use today. Most are far from faultfree. Consistent high quality results are still matters for the well trained specialist. The technical excellence of most of the electron micrographs in evidence attests to their painstaking skill. Although high-resolution electron microscopy is required for the elucidation of macromolecules, much of the experimentation is concerned with structural details just beyond the resolution of the light microscope. There has been a steady shift of emphasis from problems related to instrumentation to problems related to specimen preparation. Mastery of these elements in electron microscopy is only prerequisite, however, to the more discriminating aspect of the problem—namely, the valid interpretation of the observed microstructure with regard to concepts concerning processes of growth, degradation, and function. The information and confidence gained from properly planned and controlled experiments combining the electron microscopical approach with other independent methods of analysis have resulted not only in confirming many present ideas but in revising and filling in gaps in existing knowledge.

MEETINGS AND SOCIETIES

The scope and quality of meetings held by an increasing number of societies devoted to the science of electron microscopy provide good indications of recent developments. Annual conventions of national organizations were held in England, Germany, Japan, and the United States. Local regional meetings within the various countries are held with regularity to satisfy the wide interest in electron microscopy.

The Annual Conference of the Electron Microscopy Group of the Institute of Physics was held at Birkbeck College, University of London, on Nov. 10 and 11, 1953. As with previous conferences of the British group, papers were read concerning the electron microscope and a wide range of its applications. Twenty-three papers were given reporting on electron optics, chemical applications, reflection microscopy, carbon, silica, and plastic replica techniques, thin sectioning, and particulate structures in biological materials. The proceedings were summarized by Challice (*38*). This same group held its 1955 conference on July 5 to 7 at the University of Glasgow. Forty-one papers were read: 16 on instrumentation and general techniques, four on crystals, plastics, and air pollution studies, and six on metallurgical applications.

The 6th meeting of the German Society for Electron Microscopy was held in Münster, Westphalia, March 28 to 31, 1955. Eighty-nine papers were presented. Six general survey papers from England, Germany, and Holland were read in the opening session. Later sessions included 13 papers on techniques of electron-optical apparatus, 11 on theoretical and experimental investigations of electron optics, 23 on general problems and preparation techniques, 16 on investigations in chemistry, metallurgy, and technology, 11 on biological fine structure, and nine on virology and bacteriology. As in the past, *Physik Verhand* will probably publish abstracts and full reports will appear in *Optik* and *Zeitschrift für Wissenschaftliche Mikroskopie und für Mikroskopische Technik.*

At the 12th annual meeting of the Electron Microscope Society of America held at Highland Park, Ill., Oct. 14 to 16, 1954 (*70*), 82 papers were contributed, along with many invited discussions. Nine sessions were held, including instrumentation and specimen-preparation techniques, general biology and virology, and crystal growth in chemical and metallurgical applications. A number of special symposia were held on problems involved in the electron microscopy of viruses, electron metallography, electron diffraction, electron microscopy in petroleum technology, and industrial and applied microscopy. Because publication of many industrial investigations is restricted, symposia such as these provide a forum for exchange of ideas.

The 13th annual meeting of the EMSA was held at Pennsylvania State University, Oct. 27 to 29, 1955 (*71*). There were only 37 contributed papers and three symposia, which gave more time for spirited discussion concerning interpretative aspects of the studies. In this as in the 1954 meeting, the number of biological papers was not very much greater than those dealing with nonbiological materials. In addition, there were symposia on electron optics, emission microscopy, and preservation and interpretation of structure in biological materials. There were no papers on work done with reflection electron microscopes and only three papers on x-ray shadow microscopes. The British seem to be doing most of the work in both of these fields.

A Symposium on X-Ray Microscopy and Microradiography sponsored by the International Union of Pure and Applied Physics has been announced for Aug., 16 to 21, 1956, at the Cavendish Laboratory in Cambridge, England. The symposium will include all microscopical methods which utilize x-rays. Sessions are planned on the reflection, the contact, and the projection methods, and on applications in medicine and industry.

The continued European interest in the theoretical and practical aspects of microscopy is further evidenced by the International Colloquium on Recent Techniques in Electron and Corpuscular (Particle-Ion) Microscopy held April 4 to 8, 1955, at Toulouse, France. Twenty-six papers on high resolution, contrast, scattering, instrumentation, proton *vs.* electrostatic microscopes, and related subject matter were presented by authorities from England, France, Germany, Holland, Japan, Sweden, and the United States. The proceedings are to be published in French. The conference was sponsored by the Minister of National Education, Centre National de la Recherche Scientifique, 13, Quai Anatole-France, Paris.

The most significant meeting indicative of the growth and scope of electron microscopy was the International Conference on Electron Microscopy held in London, England, July 15 to 21, 1954 (*130*). This meeting was sponsored by the Joint Commission on Electron Microscopy set up by the International Council of Scientific Unions with membership from the Unions of Physics, Chemistry, Biology, and Crystallography to provide a basis for the exchange of knowledge in this rapidly expanding field. The success of this conference can be measured by the large number of original research papers that were presented. Of the 163 papers that were offered, 46 came from Britain, 26 from Japan, 23 from France, 23 from Germany, 15 from the United States, eight from Holland, eight from Sweden, four from Switzerland, three from Austria, three from Australia, two from India, one from Belgium, and one from Canada. Of these 70 dealt with biological subjects, 39 with instruments, 23 with metallurgical problems, 18 with electron optics, and 13 with industrial and chemical applications. Publication of the proceedings is in process (*130*). The success of this meeting demonstrated the need for periodic international gatherings and the growing need for permanent, continuing collaboration among the centers of electron microscopy. As a result, an International Federation of Electron Microscope Societies was formed in 1955. Biannual regional meetings are planned with an international conference every fourth year. The next regional meeting will be held in Stockholm, Sweden, on Sept. 17 to 19, 1956, and the 1958 international meeting is planned for Aachen, Germany. It is hoped that this international collaboration will bring about coordinated communication in a subject that touches on almost all branches of pure and applied science.

JOURNALS, BOOKS, AND REVIEWS

In January 1954 the Rockefeller Institute for Medical Research published the first number of the *Journal of Biophysical and Biochemical Cytology.* This bimonthly publication is intended to provide a common medium for the presentation of morphological, biochemical, and biophysical studies of the structure of cells and their components and of the functions of these components. Investigations dealing with cellular organization at colloidal and molecular levels derived from the newer approaches to cytology are favored. The editors will attempt to integrate information obtained from histochemistry, cytogenetics, cytochemistry, electron microscopy, and x-ray diffraction.

The publication of "Precis d'Optique Electronique" under the direction of Pierre Grivet was announced by Bordas, Paris, France. Volume I, "Les Lentilles Electroniques" by P. Grivet, M-Y. Bernard, and A. Septier was due in April 1955. Volume II, "Microscopes, Diffractographes, Spectographs de Masse et de Vitesse" by P. Grivet, M-Y. Bernard, E. Bertein, M. Gauzit, and A. Septier was planned for the end of 1955. Volume III, "Accelerateurs de Particules" by P. Grivet, M-Y. Bernard, E. Bertein, J. Seiden, and A. Septier is to be expected early in 1956.

Le Poole's doctoral dissertation (*147*) constitutes a 93-page monograph of theoretical and practical significance in electron and ion optics. Chapter I describes how, by the application of magnetic lenses, the size of electron diffraction patterns can be made independent of the wave length associated with electron beams. Chapter II deals with the principles which have led to a simplified electron microscope. Using lenses of extremely short focal length, chromatic aberration is reduced by a factor of 5 compared to conventional microscopes. A resolving power of 70 A. can be obtained even with high voltage fluctuations of 1%. Chapter III discusses the difficulties involved in obtaining accurate focus, means for getting sufficient accuracy, and a method for determining astigmatism in the objective lens. In Chapter IV a mass spectrograph with two rotating electric fields is described.

Haine (*105*) has contributed a critical review about the electron microscope. His informed evaluation and analysis of developments and problems concerning the instrument encompass 14 topics: resolving power, image contrast, the magnetic objective lens, electrostatic lenses, possible methods for the correction of spherical aberration, astigmatism, the projector lens, the electron gun, the object stage, alignment and focusing, vacuum, special features incorporated in some practical designs, and other forms and modifications of the electron microscope. Haine's review concentrates mainly on the presentation and explanation of results rather than on the methods for their derivation. Although little mathematical theory is included, the chapter is most useful to the user and designer of electron microscopes. The 140 references deal with the instrument rather than its application. The main part is concerned with the magnetic transmission-type instrument, as this form is still the most important and most widely used. The present state of knowledge about the instrument is considered rather than a review of the stages in its development.

On the other hand Hamm has written a chapter on electron microscopy (*109*) more concerned with the applications of the instrument. Preparation of specimens, the specimen-image relations, and the interpretation of electron micrographs of organic materials are described. Emphasis is placed on the interaction of electrons with the specimen and the evaluation of the factors that account for the formation and quality of the image. Alterations in the specimen leading to erroneous interpretations are discussed, together with techniques to avoid them. Application of the methods of electron microscopy to fibers, pigments, plastics, greases, soaps, crystal growth, and vapor condensates is well illustrated with 25 micrographs. Many of these methods are also applicable to problems dealing with inorganic materials. This valuable chapter with 84 selected references should assist chemists, physicists, and biologists in understanding and evaluating the present state of the art and the science of electron microscopy.

Cosslett reviewed the most recent advances in lens design and commercially produced electron microscopes. The survey includes 51 references to instrumentation, specimen preparation, reflection microscopy, and applications in biology, metallurgy, and chemistry (*43*).

Cosslett (*42*) has compared the practical limits of x-ray and electron microscopy. Nixon (*173*) has reviewed the three forms of x-ray microscopes. His discussion, including 102 references, traces the development of the reflection, contact, and projection x-ray microscopes and considers the merits and limitations of each type. At present x-ray microscopes have attained a resolution of only 1000 A.—100 times worse than that of electron microscopes. The main advantages, however, are to be found in the penetration of thick, opaque metals and living biological specimens. The selected area x-ray diffraction in combination with x-ray microscopy offers the possibility of detailed identification of chemical compounds in areas 1 micron square. Some analysis of the chemical elements may be made from a consideration of specific x-ray absorption by the different parts of the specimen. Developments leading to a very fine focus electron beam generating x-rays from a small area on the target of an end window x-ray tube have allowed Cosslett and Pearson (*45*) and Nixon (*172*) to attain resolutions of the order of 1 micron on biological specimens.

BIBLIOGRAPHIES

Because electron microscopy cuts across many of the classical divisions in science, its results are published in an ever-increasing number of articles, books, and periodicals. This dispersion and profusion of literature have become so vast that gaining ready access to this accumulated knowledge presents a real problem. Perhaps through the International Federation of Electron Microscope Societies a unified, improved means can be developed for disseminating knowledge of electron microscopy. In the meantime compilation of current literature continues in various centers.

Von Borries and Ruska of the German Society for Electron Microscopy are continuing the publication of bibliographies in its official journal (*247*, *248*). The Royal Microscopical Society (Tavistock Square, London, England) is still operating an abstracting scheme. The bibliography service offered by the New York Society of Electron Microscopists (2 East 63rd St., New York 21, N. Y.) is still available. Quarterly issues of current literature is published in the form of edge-notched cards (3.3 × 7.5 inches) punched according to both subject and author. About 1000 new cards were issued in the years 1954 and 1955. Since 1950 the compilation of recent literature in electron microscopy consists of more than 3500 references pertaining to electron microscopy, its techniques, instrumentation, and applications.

TECHNIQUES AND APPLICATIONS

NONBIOLOGICAL

One of the most significant improvements in the technique of forming substrates and replicas has been the amorphous condensed carbon film developed by Bradley (*32*, *33*). Evaporated films of carbon are very thin and very stable. They are resistant to electron beam bombardment and most chemical solvents. Replication processes are simplified and the final resolution can be better than 50 A.

Valid interpretation of electron microscopical images and evaluation of the factors which limit resolution can be aided through an understanding of what happens when an electron beam interacts with a specimen. Kanaya (*133*) obtained an analytical expression for the temperature distribution of a specimen on a thin substrate over a circular opening in the electron microscope as a function of the ratio of the radiation energy to that of conduction. When a conductive material such as gold is supported on a thin collodion substrate, its temperature is the same as that of the substrate and lower than that of the collodion alone. In the case of a protein specimen on a collodion substrate, the temperature of the specimen is higher than that of the substrate and also higher than that of collodion alone. No account was made of the accelerating potential and the amount of energy absorbed by the specimen. No specific temperatures were measured.

Astigmatism, and spherical, diffraction, and chromatic aberrations are four of the main reasons for image defects in electron optics. With 5 A. appearing as a practical limit to the conventional electron microscope, chromatic aberration caused by the interaction of a monochromatic beam of electrons with the substance of the specimen has a fundamental influence on resolution. Marton, Leder, and Mendlowitz (*154*) have investigated this process from the standpoint of the energy distribution of electrons issuing from the average specimen. They find that a surprising number of electrons lose as much as 50 e.v. of their initial energy. When these inelastically scattered electrons equal the number of elastically scattered electrons, the worst condition arises, because the optimum image focus lies between the foci of two or more different velocities. The remedy for such aberration may lie in the use of filters which would cut off the low and high velocity electrons, or in the use of lens arrangements proposed by Le Poole (*147*).

Transmission. In the past 2 years, the most work on inorganic solids has been concerned with metals and alloys. A comprehensive review of electron metallography, specimen preparation, interpretation, and typical structural formations has been made by Habraken (*102*). Menter (*159*) has surveyed the uses and limitations of electron diffraction in the study of metals.

Work on electron metallography of steel has been continued by American Society for Testing Materials Committee E-4, Subcommittee 11, with emphasis on interpretation of the bainite and martensite structures. Progress report 4 (*6*) of that committee concluded that the carbides in bainite and in tempered martensite were very similar in appearance. In both cases, the carbides tend to grow longer and parallel at lower tempering temperatures (800° F.) and to spheroidize at higher temperatures (1100° F.). The committee has also extended its studies to nonferrous alloys with a titanium-manganese alloy under investigation but still in a preliminary stage. In addition to the committee reports, individual members of the ASTM group have made further interpretation of the first stages of tempering of martensite. Fisher (*82*) has used his "extraction replica" technique to show that the first stages of carbide formation consist of epsilon-carbide and Fe_3C forms only after further tempering and at higher temperatures. Austin and Schwartz (*8*) believe there may be other percarbides besides epsilon formed on initial tempering, based on careful electron diffraction correlation with electron metallography. Teague and Ross (*240*) have also observed initial tempering of martensite with similar conclusions. In addition Ross, Sernka, and Jominy (*209*) have studied steels of various compositions and concluded that the appearance of pearlite and bainite is largely independent of alloy composition. They also conclude the reactions of austenite-pearlite or upper or lower bainite all have the same form, in that ferrite plus carbides are the products in each instance, the only difference being in the mode of carbide precipitation.

Examination and interpretation of minor phases in heat-resistant alloys have been facilitated by double-etching techniques (*25, 35*). After the initial etch which brings out both small precipitates and minor phases, a second specific etch is used to attack what Brockway and Bigelow (*35*) term the γ' phase, which is important in determining properties during high temperature aging. In this way γ' is determined, whereas it would otherwise be indistinguishable from carbides and nitrides.

A small amount of work has been done on the effect of stress on microstructure. Kehl and Bhattacharya (*137*) in a study of 1085 and 4340 steel reported no noticeable difference in the bainite structure, whether the steels were under 60,000 pounds per square inch or no stress during isothermal transformation. On the other hand, Williams and O'Neill (*258*) examined several steels and stated that strain caused breakup and solution of boundary carbides.

Substructure has been studied in copper by Pellier (née Delisle) (*54*), who showed that the substructure is different for single and polycrystalline specimens and changes after recrystallization on heating. She says that the size of the substructure blocks (1000 A. for the smallest) is in reasonable agreement with theoretical predictions and some x-ray results. Hunter and Robinson (*129*) have observed substructure in aluminum and its alloys; they state that the appearance is dependent on purity, orientation, and heat treatment. In general, the long axis of the substructure blocks seemed to be normal to (100) crystallographic planes. Berghezan (*18*) has also studied substructure in aluminum alloys and claims that the striations are parallel to (111) crystallographic planes. Bussy (*37*) states that aluminum substructure appearance is different in different crystallographic planes.

In evaporated films of aluminum-copper (*239*) oriented phases corresponding to $Cu\text{-}Al_2$, Cu-Al, $Cu_9\text{-}Al_4$ were observed and checked by electron diffraction. Bismuth films (*124*) were found to recrystallize rapidly when irradiated by the electron beam.

Gulbransen, McMillan and Andrew (*101*) have studied the oxidation of Armco and Puron in the 650° to 850° C. temperature range under pressures of 0.1 to 2 microns of oxygen. They used the oxide layers supported by Parlodion and found that the oxide crystals grow rapidly to a mosaic of large crystals. They also found that in iron containing an appreciable quantity of carbon, the oxide film reacted rapidly with the carbon, leaving a bright surface. Films formed on polished copper, nickel, and gold (*162*) by heating in vacuum (in hydrogen for the nickel) were found to be CuO, NiO, and pure gold.

Cowley (*46*) has examined the oxide layer formed by heating copper in air at 600° C. He showed that the intensity anomalies which differentiate the so-called CuO′ electron diffraction pattern are not due to impurities, as has been suggested by Gulbransen and McMillan. The oxide grows in the form of long needlelike spines approximately perpendicular to the copper surface, with one or more screw dislocations along the axis of each spine. Each spine is a single crystal of CuO, about 1000 A. in diameter, elongated along the (110) zone axis. Intensity anomalies of the CuO′ pattern may, therefore, be explained in terms of this particular morphology of normal CuO crystals resulting from growth about screw dislocations.

Minute observations of the progress of change in a solid substance undergoing physical or chemical treatment is possible by means of electron microscopy combined with selected area electron diffraction. Sasaki and Ueda (*213*) have studied the alteration in a single crystal of MoO_3 successively reduced with hydrogen for various periods of time ranging from 5 to 30 minutes at temperatures ranging from 500° to 700° C. In a certain stage of reduction atomic rearrangement can occur without affecting external shape. Thus the diffraction pattern can show profound changes without corresponding changes in the morphology observed in the microscope. Microdiffraction patterns of the reduced single crystal of MoO_3 showed some preferred orientation of MO and MoO_2 particles produced within the crystal.

Similarly, Hashimoto (*114*) has shown that besides the known transformation of MoO_3 to MoO_2 resulting from electron beam bombardment in vacuum another type of reduction occurs:

$$MoO_3 \rightarrow MoO_2' \rightarrow MoO_2$$

The intermediate phase was a monoclinic form differing from the end members.

Using microscopy and microdiffraction, Suito and Uyeda (*233, 234*) related the striped extinction patterns and the lattice structure of 3-micron single crystals grown from gold sols. The thickness of the crystals varied from 30 to 120 A. These values were determined from the deviation of subsidiary diffraction spots from the conditions imposed by the Bragg equation.

Hibi (*116*) and Yada (*119*) have studied imperfect crystals of metallic oxide smokes of molybdenum and magnesium. Unusual twins, elongations, and steps were observed in the crystal formations. The crystal habit (*118*) and the interference fringes association with magnesium oxide crystals (*117*) were further explained.

The structural and morphological properties of sols and gels have always been of interest in chemistry. Watson, Parsons, Vallejo-Freire, and Souza Santos (*249*) have made an x-ray diffraction and electron microscopical analysis of the so-called Willstaetter's C-alpha, C-beta, and C-gamma gels of aluminum hydroxide, of special interest because of their adsorptive properties for enzymes and viruses. Different structures result, depending upon the method of preparation and the age of the gel. A sol and gel prepared by the same method, however, give identical x-ray diffraction patterns and the same morphologies in the electron microscope.

Suito and Takiyama (*232*) have analyzed vanadium pentoxide sols made by two different methods. The sol prepared by cation exchange contained acicular crystals which did not grow upon aging. On the other hand, the so-called Blitz sol grew into orientated fibers upon aging.

The relationship between color and the particle size of gold sols has been ascertained (*160, 244*).

A considerable literature has been established concerning the chemical composition and physical state of the compounds produced in the hydration of portland cement. Fundamental understanding of the process has been hampered by the lack of information regarding the size, shape, structure, and crystalline nature of the poorly defined gelatinous substances and other phases which make up the dynamic colloidal system during initial, intermediate, and final stages. Electron-optical methods in conjunction with x-ray diffraction, specific adsorption measurements, and chemical analysis have been recently applied to these problems (*19, 26, 100, 236*). Although the results of these investigations have been advanced mainly as preliminary or progress reports, present interpretations not only provide data on the morphology and crystalline state of the products of hydration but suggest the nature of the mechanism of hydration and the cementing action of portland cement.

Monodisperse latices are important in investigating the mechanism of emulsion polymerization. They have also gained importance as secondary calibration standards in light and electron microscopy, light scattering and sedimentation studies, and aerosol studies. Bradford and Vanderhoff (*31*) have prepared monodisperse latices with particle diameters ranging from 1000 to 12,000 A. Polystyrene, polyvinyltoluene, and various copolymers have been formed from carefully controlled emulsion polymerizations. A series of polystyrene latices is available for distribution. The variations of particle diameter resulting from different substrates, electron irradiation, and other sources of error were statisticaly evaluated.

Kienle and Maresh (*138*) have made observations on pigmented films. By means of replicas, thin sections, and studies of particle size, shape, and dispersion, they have provided a better understanding of the causes of and the changes in the surface reflectance of paints and coatings.

Kling (*141*) has contributed to the understanding of the theory and mechanism of cleaning. By using a replica technique Kling and Mahl (*142*) have shown that the distribution of soil particles in cotton and wool fibers is surprisingly uniform. Gotte, Kling, and Mahl (*95*) have shown that the number of dirt particles on the fiber surface is directly related to the lightness of shade of the textile. Coarser particles are more readily removed. Particles smaller than 2000 A. become more difficult to remove, but migration of the dirt into the interior of cotton and wool fibers apparently does not take place.

Rollins (*207*) has reviewed the use of light and electron microscopy in the study of the gross and fine structure of native cellulose fibers. Rollins and Tripp (*208*) have reported their studies of cotton fiber structure and Tripp, Moore, and Rollins (*243*) have studied the effects of some typical chemical environments on the isolated primary wall of the cotton fiber. From these and structural considerations properties of interest to the textile technologist are discussed.

Peck and Kaye (*190, 191*) have developed a technique for etching cellulose acetate fibers and films by direct solution of the surface molecules. A relatively undisturbed interior layer of polymer is uncovered for replication. Etching is achieved by immersing the polymer for a short time in acetone at −50° C. and then flooding with an excess of cold absolute alcohol. Replicas reveal the orientation of molecules in the skin, the existence of voids, and pigment dispersions in cellulose acetate yarns. Modifications of this technique will undoubtedly arise as new plastics are studied. Through the use of aluminum-beryllium replicas, abraded surfaces and the skin of cellulose acetate fibers were studied. It was found that ceramic guides for the fiber cause a sticking action, whereas metallic guides produce a shearing of the surface. The analysis aids in the quality control of the production of synthetic fibers.

By reflection electron microscopy Chapman and Menter (*40*) have studied directly the shape, surface structure, and frictional wear of small fibers of Orlon, viscose rayon, cellulose acetate, glass wool, and undrawn and drawn nylon. In the case of single fibers of drawn nylon, they found that under high sliding pressures the fiber undergoes considerably permanent deformation and severe tearing occurs. To overcome the low beam intensity used to prevent damage to the specimen, a large angular aperture is needed in the objective lens. This and specimen motion reduce resolution to about 1000 A. Although such resolution is only slightly better than with the light microscope, the great depth of field and the low viewing angle permit a large part of the curved surface to be seen.

By means of autoradiography and electron microscopy Hock (*120*) has obtained new information about the form and distribution of rosin sizing on a sheet of sized paper. His observations support the often expressed opinion—hitherto not adequately confirmed—that rosin size is not present as smooth surface-covering films, but rather in the form of discrete particles about 1000 A. in diameter and that they occur either singly or in groups.

Insight into the structure and behavior of leather may be gained from studies by Swerdlow and Stromberg (*237*). The existence and morphology of pores of the order of 150 A., in radius, within collagen fibrils were delineated. Structural features of collagen from air-dried kangaroo tail tendon, impregnated with mercury at a pressure of 10,800 pounds per square inch, were compared with those of fibrils not exposed to mercury. A helical configuration of subfibrillar elements was suggested by the electron micrographs of individual fibrils. The possible use of mercury under hydrostatic pressure as a technique in staining and preserving the structure of biological materials was advanced. These findings constitute a visual confirmation of the presence and probable size of small pores deduced from theoretical considerations of pore-size distributions in collagen and leather, and provide additional information about the shape, location, and arrangement of such pores in collagen fibrils.

Antal and Weber (*7*) have reported an electron-optical study on the surface of glass. By depositing a thin film of bismuth on the surface of glass, they were able to overcome surface charging and obtain electron-diffraction patterns of the substrate-glass. A calculation of the radial distribution function for a soda-lime-silica glass surface indicated a deficiency of alkali atoms in the topmost atom layers, which is attributed primarily to water washing of the surface prior to examination.

Terao and Sakata (*241*) have done some work on fresh fractures in glass, where the starting point of cracks was observed. It is claimed that artifacts due to reaction of the replica and the glass did not interfere with observation of the real structure.

Direct electron microscopical evidence of structural inhomogeneities in glass has been reported by Prebus and Michener (*198*). Among several glasses investigated a grainy structure ranging from 20 A. up to at least 200 A. has been observed. These have been interpreted as micellar regions which have a degree of order greater than that of the short-range order assigned to the glassy state by the random-network theory, but less than that of the long-range order characteristic of the crystalline state. Further study by the methods of electron optics and radiation scattering on glasses of known composition and thermal history is needed. An investigation of nucleation and crystallization phenomena at or near the liquidus region would lead to a better understanding of the process whereby a liquid changes into a crystalline mass. From such studies further insight into the glassy state of matter might be obtained.

Thermionic Emission. Interest seems to be increasing in this type microscopy for studying metal surfaces directly at elevated temperature. Rathenau and Baas (*199*) described instrumentation and activation of metal surfaces (such as iron-carbon alloys) with evaporated layers of barium, strontium, or cesium.

They have shown examples of application to grain growth studies, interfacial angles and energy, and phase transformation. Septier (*221*) and Menter (*157*) have described applications to metallurgy, and Menter has cautioned that the free surface examined by emission microscopy may not look the same as the internal volume of the specimen—this is important in phase transformation. Heidenreich (*115*) has published a careful study of the elements which promote or poison emission from various metals; he finds carbon to be the most effective promoter for copper, nickel, and iron. He uses barium formate for activation and agrees with Baas that one should, if possible, use pure iron-carbon alloys rather than ordinary steel because the impurities poison emission below 700° C. He proposes a simplified mechanism for all the transformations from austenite; first $\gamma \rightarrow \alpha' + \gamma_{\text{retained}} + \gamma_{\text{recrystallized}}$ in which the α' is unstable and transforms $\alpha' \rightarrow \alpha$ + carbides. Thus the same mechanism covers both high and low temperature transformation. Mollenstedt and Hubig (*161*) have discussed the film formed on metal surfaces by ion bombardment such as may exist in an emission microscope. Unless the metal is kept above 150° C., a structureless film will form on it in a partial vacuum (say 10^{-3} mm. of mercury); once formed this film is not removed by heating, and it obscures the structure one is trying to see.

Reflection. Attention to reflection microscopy has increased the past few years, especially in England. In general, the microscope is the same as for transmission, but the beam is inclined to strike the solid specimen at grazing incidence (less the 10° angle); this results in higher magnification in one direction than in the other. Menter (*158*) has shown applications to the study of cleavage surfaces on mica, growth steps, twinning, etc. Resolution of about 200 A. is claimed. Cosslett and Jones (*44*) described a variable incidence instrument in which electron diffraction could be accomplished without moving the specimen. Jones (*131*) developed a stage for heating the reflection specimen to 1000° C. while observing the image.

As in other techniques, Fert, Marty, and Saporte (*81*) employ an electron beam with an illuminating angle of a few degrees, but the viewing angle is 25°. This reduces distortion and the extent of foreshortening of the enlarged image. With a narrow incident beam and a lightly etched specimen, surface relief of 20 A. is detectable. An extra gun for continuous ion bombardment is necessary to reduce surface contamination. A resolution of about 350 A. is claimed. Haine and Hirst (*106*) using illuminating and viewing angles which total 6° to 10° attain a resolution of about 430 A.

A different approach in which the specimen acts as a "mirror" in a reflection electron-optical system has been used by Mayer (*155*) and by Bartz, Weissenberg, and Wiskott (*15*). In this work the specimen surface is normal to the electron beam and what are actually observed are differences in potential on the surface. Surface irregularities as well as composition changes act as sources of potential difference, so the image is similar to the usual surface micrograph. A resolution of 3000 A. is claimed by Mayer (*155*), but he states that the theoretical limit should be at least as good as with thermal emission electron microscopy.

A new technique using the x-ray–sensitive properties of crystals or "rigid vinyl" plastic film allows relief images to be formed in x-ray microradiography (*146*). Replicas of these relief images can be observed in the electron microscope at useful magnifications of 10,000 to 25,000 diameters. This technique is promising because it makes use of the penetrating power of x-rays and the resolution of the electron microscope.

BIOLOGICAL

Technical advances have continued at a rapid pace during the past few years in practically all aspects of the technique for preparing biological material for examination with the electron microscope. The addition of low concentrations of silver nitrate to the drinking water of experimental animals for long periods results in the development of a vital stain as well as an electron stain of sorts, the silver, possibly in the form of a silver proteinate, being deposited in basement membranes of various glands, of renal glomeruli and proximal convoluted tubules, and of vascular endothelium. Intracellularly it was found in fixed and free machophages in various locations (*59*). With the exception of the choroid plexus, area postrema, (*60, 246*) neurohypophysis, and pineal body (*60*) in which the reaction is in general like that in other organs of the body (*59*), little or no silver was found to be deposited in the central nervous system (*60, 246*). These results do not necessarily imply that the penetration of ionic silver is correlated with silver deposition (*246*).

Studies are at present under way in several laboratories, the purposes of which are to develop new histochemical methods or adapt previously established histochemical techniques for use in electron microscopy. At this writing one article demonstrating the possibility of studying the sites of acid phosphate concentration (*222*) has appeared as the result of such studies. Another has demonstrated that methyl mercuric chloride reacts specifically with protein-bound sulfhydryl groups to produce electron-dense areas in, for example, squamous epithelial cells involved in the process of keratin formation (*11*).

The problem of fixation for electron microscopy remains one of primary importance. The veronal-acetate–buffered osmic acid fixative of Palade (*182*) remains the fixative of choice in the majority of laboratories and continues to give superior results. A chrome-osmic fixative (*47*) has been suggested as a useful adjunct to Palade's fixative and has been recommended as particularly useful for studies on the central nervous system (*153*). A chrome-formol fixative has been used for comparison with osmic acid to determine how much detail is visualized as the result of the deposition of osmium (*151*). There is a definite need at the present time for a fixative not containing osmic acid, which will give equally faithful preservation of cell detail (*51*). One of the well recognized difficulties associated with the use of osmic acid is the determination of the degree of satisfactoriness of fixation. Because of the slowness of penetration it may frequently be observed in blocks of tissue fixed with osmic acid that of two immediately adjacent cells, one may appear well preserved and the other poorly preserved. It is therefore necessary to consider this factor very carefully if the results of experimental intervention are to be interpreted correctly. In the present situation it is essential to establish criteria for identifying good or bad fixation when either is present. Since osmic acid or fixatives containing osmic acid are the only generally acceptable fixatives for electron microscopy of tissue sections, the studies of Bahr (*9, 10*) on the reaction of osmic acid with various chemical constituents of protoplasm are of considerable importance.

While little advance has been made in the area of fixation *per se*, there is some evidence that treatment after fixation may be of value in certain instances. Phosphotungstic acid has been used to increase the contrast of viral particles (*107*) and fibrous structures (*218*) and uranium nitrate has also been found to increase the contrast of certain virus particles (*132, 238*).

All those who have carried out moderately extensive studies on the electron microscopy of tissue sections using methacrylate must have observed polymerization damage. Such damage may vary from extreme disruption in which even nuclear and cytoplasmic boundaries are unidentifiable, to minor effects such as occasional artificial interruptions in membranes bounding the nucleus, cytoplasm, mitochondria, etc. Polymerization damage, varying from block to block frequently makes interpretation of experimental intervention very difficult. Damage of this sort is less noticeable in tissue blocks, especially if the original blocks are small, but may be particularly severe in preparation of protozoa or cells of the peritoneal fluid, of blood, and of cells in tissue cultures. It has been found, other things being equal, the substitution of benzoyl peroxide for Luperco (2,4-dichlorobenzoyl peroxide) will reduce polymerization damage (*125*). Prepolymerization to a viscous state before introduction of the tissue will reduce polymerization damage under certain conditions (*28*). This practice is particularly valuable in obtaining final embeddings of the same consistency. Further, double embedding, first in 2% agar in 10% formalin immediately after fixation, followed by the usual dehydration and embedding in methacrylate, greatly decreases polymerization damage of free cells and of cells on free surfaces (*78*). In addition, cells embedded in agar after fixation are handled as tissue blocks, eliminating the need for repeated centrifuging. Borysko (*27*) has demonstrated the value of high temperature embedding (60° to 80° C.) in reducing polymerization damage in tissue culture and embryonic cells. Karrer (*134*) has demonstrated its value in reducing damage to cells on free surfaces (pulmonary alveolae and bronchioles).

As for the embedding medium itself, various proportions of methyl and *n*-butyl methacrylate, from 1 to 4 (*217*) to 1 to 20 (*94*), are used to obtain the proper final consistence of the polymerized plastic. Apparently the use of large-sized (00) gelatin capsules as containers results in hardening of the polymer to a degree which makes the addition of methyl methacrylate unnecessary. As far as thin sectioning is concerned, the glass knife continues to be the cutting edge of choice in the majority of laboratories, although the razor blade is still producing satisfactory results for Sjöstrand (*229*). The possibilities of a polished diamond edge (*80*) are beginning to be explored (*79*). Modification of existing microtomes (*56, 93*) and newly designed microtomes (*195, 227*) are producing consistently satisfactory results. New additions to this group (*80, 225*) appear to be equally satisfactory.

Serial sections, while difficult to obtain, have been shown to be of value in determining the finer structure and interrelationship of cell components (*88, 164, 195*). Athene screens (Smethrust High-light, Ltd., Sidcot Heaton, Bolton, Lancs., England) of various designs are useful for this and other more general purposes.

Sectioning artifacts—that is, loss of tissue by sectioning, modification of structure resulting from imperfection of the cutting edge and from compression during cutting—as well as artifacts induced by exposure to the electron beam have recently been emphasized (*167, 260*). Knife marks originally evident at low beam intensity may be modified or completely hidden by the flow of plastic occurring under high beam intensity. It has also been demonstrated that substances that are incompletely denatured by fixatives such as formalin may be sublimed off and lost as a result of exposure to high beam intensity (*169*).

Carbon supporting films have been found to eliminate specimen drift and to have about the same electron-scattering properties of the usual Formvar films. Such films specially prepared to possess small openings allow the examination of completely unsupported tissue for high resolution electron micrography (*250*).

Undoubtedly the majority of investigators have experienced the difficulty of finding brush marks, glass chips, imperfections in the emulsion, etc., on their best negatives. Antiabrasive plates treated to resist exposure to high vacuum are available (Eastman Kodak Co., Rochester, N. Y.) as well as fine-grained spectroscopic plates. It would seem reasonable to use plates of these types, even though greater expense is involved, since the photographic negative is the final result of all the preliminary effort.

Several attachments which improve the efficiency of the RCA EMU 2 series electron microscopes are available commercially. One of these is the externally centerable condenser lens aperture allowing a choice of four aperture sizes. (Canal Industrial Corp., Washington, D. C.). Another is the deflection beam focuser, which greatly improves accuracy of focusing (Canal Industrial Corp.). A third is the below-focus objective aperture, of particular value because in this position there is less likelihood of contamination (Canal Industrial Corp. and Illini Associates, Urbana, Ill.).

Some of the results recently obtained by the application of these technical methods will now be considered in the following order: (1) virus studies, (2) bacterial morphology, (3) the structure of plant cells, (4) general cytology of animal cells, and (5) special cytology of animal cells.

Virus Studies. A good deal of the recent work in the virus field has been accurately and adequately reviewed by Bang (*12*). Only the pertinent papers which have come to attention since Bang's review are considered here.

Before considering these articles, however, it will do no harm to re-emphasize the importance of utilizing the criteria described by Bang for identification of a virus as those particles seen by electron microscopy: (1) characteristic appearance either as individual particles or as groups of particles, (2) association of the agent with disease or infection, (3) physical testing of the association of particles with the infectiousness of the preparation, (4) association of particles with other known activity of the virus, (5) agglutination of the particles by immune sera, preferably sera from convalescent animals, and (6) infection produced by a very few particles. It is important to bear these criteria in mind when evaluating any claim regarding the identification of a particle seen in sections with the electron microscope as a particular virus.

It is generally agreed that when observed in thin sections, cells infected with virus contain particles which are elliptical or spherical in form and possess a central electron dense core and a single or double peripheral membrane separated from the core by a less dense region. The size of these particles varies from 500 to 5000 A. Cells infected with *Herpes simplex* virus contain primary bodies 300 to 400 A. in diameter and somewhat larger (400 to 500 A.) bodies covered by a peripheral membrane—both within the nucleus. Cytoplasmic particles were larger and possessed a double outer membrane 1000 to 1300 A. in diameter. These observations suggested that viral development is intranuclear and that upon migration to the cytoplasm the particles become mature (*165*). Vaccinia and fowl pox viruses on the other hand are associated with particles 2000 to 3000 A. in diameter originating in the cytoplasm. These particles possess a dense central body (nucleoid) and a single peripheral membrane. In the peripheral cytoplasm and extracellularly the nucleoid is larger and the peripheral membrane is double (*166*). Still another variant is the influenza virus. One study demonstrated that infected cells develop rods and spheres at the cell surface, not within the cytoplasm proper or the nucleus (*168*). However, in another study ciliated cells of brochii experimentally infected with influenza were found to possess cytoplasmic inclusion bodies, which on examination with the electron microscope were found to consist of particles of the size estimated for influenza virus (*110, 111*). The intracellular form of particles associated with infection with anopheles A virus has been described (*85*) and has been shown to develop in association with the membranes of the endoplasmic reticulum or ergastoplasm (*84*). In addition, particles associated with the Shope fibroma have been classified in the same category with particles seen in vaccinia and fowl pox (*22, 149*). Particles associated with the Murray-Begg endothelioma were found both in an extracellular position and in the cytoplasm. The latter particles showed continuity with membranes forming the wall of vesicles of undetermined origin (*104*). This latter relationship is possibly similar to that described in anopheles A infection (*84*) and to particles found in the cytoplasm of the Cloudman melanoma S-91 (*50*).

Particles again differing in size depending on whether they are intracytoplasmic (120 to 400 A.) or extracellular (1000 A.) have been found in mammary tumors of mice carrying the milk agent (*21*). In cells of the Rous tumor extracellular and intracytoplasmic particles have been found to approximate the same size (500 A.). The particles consisted of a thin peripheral membrane usually surrounding a dense central core (*90*). A representative of the psittacosis lymphogranuloma group, meningopneumonitis virus, has been studied, and the cells infected with it have been characterized as possessing particles of different size groups (2500 to 3000, 3000 to 4000, 4000 to 5000, 5000 to 6000 A., and a still larger form) exhibiting different internal structure. The presence of what appear to be budding forms suggests that these different particles represent different stages of a life cycle (*91*). Viruslike particles have also been found to be associated with the Ehrlich mouse ascites tumor (*217*), with Sarcoma 37 (*50*) with spontaneous hepatomas of C3H mice (*77*), and in the blood plasma of leukemic mice (*65*). Particles 500 by 300 A. in a crystallike pattern in the nuclei of cells infected with an animal virus have also been described (*140*).

A study on virus-host cell relationships has shown that certain viruses will kill the host cells (Mengo and anopheles A), while others will multiply within the host cells but will not destroy them (influenza and unadapted Newcastle virus) (*83*). A more general account and review of virus-host relationships have recently appeared (*13*).

The special cases of insect polyhedral virus disease have been investigated and the macromolecular paracrystalline lattice and associated viral particles have been described (*163*). Stages in the development of a nuclear polyhedral virus disease have also been described. Elementary virus rods proliferate within the chromatin mass of the nucleus. They are then released into the nuclear sap. Membranous envelopes develop around the rods and finally the polyhedral protein is deposited about the encapsulated viral particles (*53*). It has been suggested that the viral protein does not come directly from chromatin, although chromatin may be involved indirectly in the process of viral protein accumulation (*261*).

Obviously, not in all of these investigations have the majority of the criteria (*12*) for establishing a definite causal relationship between a virus disease and a viral particle been applied, but the background of observations which is developing should soon make possible the clarification of life cycles in some instances and the classification of virus particles on the basis of morphology in others.

Before passing on to other areas an interesting and significant observation concerning bacteriophage should be recorded. The tail and tail fibers, not the head, are responsible for the primary state of absorption of phage to bacteria (*259*).

Bacterial Morphology. Turning now to the morphology of bacteria—there have been claims in the past of the presence of formed elements of both cytoplasm and nucleus in these species. Recent evidence suggests that a nucleus of a sort containing deoxyribose nucleic acid does exist in bacteria. There is also some reason to feel that nucleoli exist. However, recent studies have produced no evidence of the presence of mitochondria in bacteria (*29*). The conservative view has also been expressed that inasmuch as what appear to be nucleoli may be cytoplasmic invaginations into a central vacuole and no membrane has been described bordering what has been called the nucleus, a strict homology with the nucleus and nucleolus of higher forms should not be made at present (*242*). There is also evidence that tonicity of the fixative and the environment immediately before fixation are important factors affecting the finer structure of bacteria (*206*). In any case at the present time it is a relatively easy matter to distinguish bacteria and structures related to the presence of bacteria in infected cells from all other structures present in uninfected cells (*34*). Investigations being carried out in several different laboratories should shortly clarify many aspects of the morphology of bacteria which are at present disputed or unknown. This is an area in which studies of shadowed preparations of whole mounts will continue to give information not necessarily readily obtained from sectioned material (*123, 144, 145*).

Structure of Plant Cells. Primary emphasis in studies of the electron microscope of plant cells has been concerned with the structure of chloroplasts and grana, although spore walls (*3*) and walls of pollen grains (*2*) have been examined. Studies on yeast cells have also been made, in which it was found that cells grown on a glucose yeast extract were useful for studies on the cell wall and on bud and birth scars. However, it was necessary to grow cells on a presporulation medium in order to obtain material in which the detail of cell membrane, mitochondria, and nuclei could be observed in these sections (*4*). Studies on chloroplasts have been carried out in Chlorella (*5*), Chlamydomonas (*212*), Aspidistra (*148*), and Zea (*122*). The results of these studies indicate that chloroplast structure becomes increasingly complex with the appearance of grana in higher forms. Chloroplasts consist of stacked layers of membranes or laminae with which chlorophyll is associated (*122*). Grana are more dense regions of chloroplasts in which the laminae become twice as thick as in the stromal portion of the chloroplast (*231*) and are approximately twice as numerous (*122*). Chloroplasts have been described as developing from a small granule in young cells. This granule separates into a central dense core and a more peripheral stromal zone. Laminae develop as enveloping membranes around the dense core. Later additional laminae appear to be derived from previously formed ones.

Mitochondria with characteristic internal structure have been described in plant cells (*212*), but further analysis and comparison of these as well as other cytoplasmic and nuclear components in relation to their counterparts in animal cells lie in the future.

Cytology of Animal Cells. The large bulk of technically excellent high resolution electron microscopy of biological materials has been carried out on animal cells. This is true in part because with this type of material satisfactory fixation, embedding, and sectioning were most readily obtained. In the past few years the broad outline of the general cytology of animal cells has been filled in. The plasma membrane with its specializations and some of its activities, the fine structure of mitochondria, the detailed structure of the Golgi complex, the small granules of the basophilic substance, the membranes of the endoplasmic reticulum, the nuclear membrane with its stomata, details of the nucleolus, nucleoplasm, and even chromosomes and the mitotic figure have all been examined—in most cases with care and precision. In the area of special cytology many differentiated cell types with their particular characteristics have been competently analyzed.

Specializations of the cell surface have been examined in a series of cell types. The striated border (*256*), brush border (*202*), and sterio-cilia of classical cytology have all been shown to consist of essentially similar microvilli. In addition to the cells long recognized as having a cuticular border, others such as mesothelial cells (*78, 175*), cells of the gall bladder (*263*), and parietal cells of the stomach (*214*), embryonic yolk sac (*55*), and chorion (*58*) have been shown to possess microvilli. Microvilli may vary considerably in length, in different cell types, but are in general characterized by having a constant diameter through most of their length and being relatively closely and regularly spaced. The characteristic structure of cilia (*30*) has been extended and clarified. The nine peripheral paired filaments and two central filaments of cilia (*76, 215*) are also characteristic of the filaments of sperm tails (*36*). Cilia, of course, cannot be considered simply as a surface specialization of cells, since ciliary roots extend some distance into the cytoplasm and in the case of sperm tails originate in relation to the centriole. However, the cell surface is involved in their development and formation and the preliminary observation (*126*) that microvilli sometimes possess the 9 and 2 arrangement of internal filaments has extremely interesting connotations. This would correlate well with the finding of cilia and microvilli on the same cells of moluscan (*76*), amphibian (*76*), and mammalian (*76, 111*) ciliated epithelium. Microvilli have been considered as of the same nature as the temporary cell processes of free cells (*216*); however, the latter are extremely variable in length, diameter, and spacing, and are frequently broadly based. Another difference is that in some instances, at least, in proximal tubule cells of the kidney (*202*), in principal cells of the duodenum (*50*), in epithelial cells of the gall bladder (*263*), and in mesothelial cells (*78, 184*), coiled tubular invaginations extend downward into the cytoplasm between the bases of the microvilli. Other examples of this correlation may come to light with more careful analysis of other cell types.

These tubular invaginations may be comparable to vesicular structures associated with the plasma membrane of endothelial cells (*179*) and with the vesicular component of Schwann and nerve satellite cells (*62*) which together on a finer scale may represent pinocytosis in these cell types. The basic process involved in the formation of these invaginations is possibly the same as that involved in the process of phagocytosis, but when melanin granules approximately 1000 A. in diameter are injected into the peritoneal cavity, only macrophages phagocytose them (*78*). When thorotrast, a much-small-sized particle, is injected, both mesothelial cells and macrophages engulf them (*176*). This suggests the possibility that different physical-chemical phenomena may be involved in the absorption of the two types of particles. Thus the problem of transport of material across plasma membranes is a complex one, involving solubility and semipermeability, pinocytosis and phagocytosis. The problem of the movement of materials in solution across semipermeable membranes can probably be better approached by means other than the electron microscope.

As indicated above, however, the electron microscope is useful in studying the mechanisms involved in pinocytosis and phagocytosis and should eventually give some evidence as to whether they are one and the same. This whole question is further complicated by the suggestion that the plasma membranes invaginated during the process of pinocytosis become part of and continuous with the membranes of the endoplasmic reticulum or ergastoplasm (*181, 254*). These membranes in turn are in continuity with the nuclear membrane (*251*) and apparently in some instances with membranes of the Golgi complex (*187*). This suggestion of the origin of intracellular membrane systems is an intriguing one [the evidence for their intracytoplasmic origin is equally good (*254*)], but a corollary to this hypothesis must be an explanation of how cells such as ameba and macrophages decide which plasma membrane to incorporate into cytoplasmic membrane systems and which to discard. Evidence from time-lapse photomicrography showing rapid and continuous incorporation of vacuoles at the cell surface certainly indicates that, if all invaginated plasma membranes were retained in the cytoplasm, macrophages would soon consist of nothing else. Actually the evidence for this relationship is at the present time mostly circumstantial.

During the past few years the details of the structure and relationships of cytoplasmic membrane systems have been clarified somewhat. The basophilic substance of cytoplasm (ergastoplasm) in its most highly specialized form has been shown to consist of a system of double membranes which are arranged in parallel rows or concentric circles (*228*). In this condition some anastomosis between rows of membranes occurs and it has been shown that these membranes are actually profiles of flattened sacs (*254*). In addition, these membranes have been demonstrated to be the cut profiles of membranes of the endoplasmic reticulum (*187*). These membranes with their associated small granules (*180*) vary greatly in number and complexity from one cell type to another (*184*). There is clear evidence now that cytoplasmic basophilia is related to the small granule component (*23, 180, 194*). It has also been suggested that they are the primary site of concentration of cytoplasmic ribose nucleic acid (*185*), although there is evidence to indicate that large amounts of ribose nucleic acid are present elsewhere in the cytoplasm (*143*). The granules may be closely associated with a highly developed membrane system as in exocrine cells of the pancreas (*228*) and cells of the parotid gland (*184*), partly free and partly associated with membranes as in principal cells of the duodenum (*194*), tumor cells (*50*), and nerve cells (*187*).

The establishment of an acceptable terminology which can be used to describe all variants and combinations of these two components, the small granules of Palade, and the membrane system is necessary but not easy. The small granules are responsible for the basophilia and the membrane system for the apparent filamentous or lamellated appearance originally described for ergastoplasm. The term "ergastoplasm" would be historically accurate and descriptively correct when applied to heavily basophilic cells such as those of the pancreas and parotid gland, but its use in describing the same components in strongly acidophilic cells such as parietal cells of the gastric mucosa would require some modification or extension of the original meaning. A similar or greater difficulty is encountered in attempting to apply the phrase "endoplasmic reticulum" to all cell types. Obviously, when the term was first coined (*196*), it applied to both the membrane system and the small granules associated with the membranes. In addition, there are many instances in which the system is not limited to the endoplasm of the cell and in other instances the membranes do not form a continuous reticulum. It is uncertain as to whether in the near future terminology can be standardized for these cytoplasmic components which will be scientifically accurate and historically correct, and will at the same time satisfy national and personal loyalties. What is certain, however, is that when the term "ergastoplasm" is used, it is used in reference to the combination of Palade granules and the membrane system (*23*) and that the term "endoplasmic reticulum" refers only to the membrane system itself (*184*).

The origin of small Palade granules is at present under investigation and discussion. The possibility exists that they originate *de novo* in the cytoplasm, although recent racer studies suggest that they originate in the nucleus and pass into the cytoplasm later. This question of origin and also the related one of continuity between nucleoplasm and cytoplasm may have been answered in recent studies on the nuclear membrane. The earlier observation that the nuclear membrane is a double structure has been confirmed in one sense and modified in another. There is no question but that two membranes exist; however, it has recently become clear that the outer of these two membranes is the inner boundary of the cytoplasm and that as such it is in continuity with the membranes of the ergastoplasm or endoplasmic reticulum (*50, 180*). It also seems certain that the two membranes become continuous at the margin of what appear to be points of continuity between nucleoplasm and cytoplasm (*50, 180, 251*). However, a thin membrane has been seen extending across these openings (*1*) and densitometric analysis of electron micrographs indicates an increase in osmiophilia at exactly the area where the openings should be (*135*). The numerous perforations described in some nuclear membranes, notably paramecium (*251*), may be suspect because of the possibility of contraction during the slow penetration of osmic acid into large cells covered by a dense pellicle. However, there would appear to be no question but what under certain experimental conditions definite modification in the structure of the nuclear membrane may take place (*112*). Unquestionably in such a dynamic system as the animal cell, some means of transport between nucleus and cytoplasm must exist, but whether these so-called nuclear pores subserve this function remains to be proved.

The fine structure of the Golgi complex was recently described in cells of the epididymis as consisting of a system of smooth double membranes, small granules or vesicles, and large vacuoles. The three components were clearly shown to be in exactly the same area as the classical reticulum in osmic impregnated cells (*49*). Since this demonstration the three basic components have been described in good detail in duodenal cells (*51, 52*), pancreas (*51, 52, 229*), plasma cells (*23, 51, 52*), hepatic cells (*52, 75, 103*), white blood cells (*24, 192*), spermatids (*36, 51, 75, 96*), cells of the hypophysis (*73, 74, 103*) and thyroid (*57*), and nerve cells (*51, 187*). In tumor cells the complex may be highly developed (*103*) or extremely small, consisting only of a few membranes and vesicles (*50, 128*). It has been suggested that because the claim of artifact has been made against the classical Golgi apparatus, because many different things have been included under the name Golgi substance, because of the variable morphology of the structures concerned, because of uncertainty as to whether this fine structure actually is the electron microscopical representation of the classical Golgi reticulum, and because of the evidence of continuity between membranes of the Golgi complex and those of the ergastoplasm or endoplasmic reticulum, a new term "agranular reticulum" should be substituted (*187*). In spite of the above objections the term Golgi complex or apparatus should be retained for several reasons. First, the label of artifact applies in no greater degree to the fine structure of this cell component than to any other. Secondly, with the resolutions obtainable with the electron microscope there is no difficulty distinguishing the Golgi complex, whether highly or poorly developed, from any other cell structures and thus there is no longer any reason for confusion. (We continue to refer to mitochondria, even through at one time they were frequently confused with bacteria, secretory granules, and melanin granules.) Thirdly, the contents of the small vesicles, large vacuoles, and the area enclosed within the membrane systems of the Golgi complex reduce osmic acid when no other components of the cell do, indicating a distinct difference between the Golgi complex and ergastoplasm (*48*). Fourthly the term "agranular reticulum" is inappropriate because it fails to include reference to the small vesicles and large vacuoles, which are clearly separate although undoubtedly derived from the membrane system of the complex.

The characteristic finer structure of mitochondria, once described (*183*), has been extended to include the mitochondria of variety of cell types. Differences in detail have been found (*51, 197, 202, 215, 228, 252*); however, with but few exceptions, the presence of a continuous outer peripheral membrane and an inner peripheral membrane extending as cristae in the form of flattended sacs or villi into the mitochondrial matrix has been confirmed. The suggestion that a continuous peripheral membrane is sometimes lacking (*197, 252*) may probably be discounted on the basis of less than adequate preservation or resolution. The suggestion that mitochondria may be involved in the intracellular concentrations of cations (*255, 257*) is an interesting one and should stimulate investigations aimed at verifying and extending it. The general area of the relationship between fine structure, function, and biochemistry of mitochondria is so large that it would require a separate review of its own. In this area the great value of electron microscopy will be as a service to biochemists in determining the degree of intactness of isolated mitochondria.

One other special function suggested for mitochondria is their direct involvement in the formation of secretory granules in pancreatic cells (*39, 86*). This suggestion brings up the general question of the relationship between secretory activity and the various cytoplasmic components. It has been indicated that under certain conditions ergastoplasmic sacs are directly involved in the formation of secretory granules in pancreatic cells (*254*). It has also been suggested that the small vesicles of the Golgi complex enlarge to form secretory granules (*229*). Electron micrographs may be produced to give evidence for any one of these three hypotheses. On the other hand, there is clear evidence that the Golgi complex is directly involved in the formation of the acrosome in spermatids (*36*), in the formation of specific granules in cells of the anterior hypophysis (*103*), and in the absorption and modification of and release of lipide material from principal cells of the duodenum (*256*). It would appear that the hypothesis which most satisfactorily takes into account all of the currently available evidence is that the Golgi complex is primarily involved in the removal of water from maturing secretory substances, on the one hand, and engulfed or absorbed material on the other (*51, 86, 87*).

Some information has recently become available regarding the fine structure of nucleoli, nucleoplasm, chromosomes, and the achromatic spindle of mitotic figures. Nucleoli, whether of normal or malignant cells, are composed primarily of a concentration of small granules similar in size and electron density to the small granules of Palade in the cytoplasm. Interspersed between these concentrations in a spongelike arrangement are less electron-dense areas containing both small granules and fine anastomosing filaments (*20, 50*). Occasionally associated with nucleoli are large areas free of granules but containing numerous anastomosing filaments approximately 70 A. in diameter. These areas may represent nucleolar associated chromatin (*50*). Chromatin bodies of the macronucleus of a protozoan have been shown to vary from a dense homogeneous sponge work in adult organisms to highly organized structures (appearing as a honeycomb in cross section and as parallel lines in longitudinal section) in old individuals (*210*). Meiotic chromosomes show a variation in granule and filament content, the filaments differing in diameter depending upon the stage of division. Very fine (28 ± 7 A.) threads are thought to represent single deoxyribonucleoprotein molecules (*66*). Thin tubular elements not encountered in resting cells are thought to represent elements of the achromatic spindle (*193*). In some meiotic chromosomes, in addition to the granular and filamentous component, a central compound core of tubules has been visualized (*170*). Serial sections suggest the possibility that nuclear blebs containing chromatin and nucleolar material may be separated off to be distributed in the cytoplasm (*89*). These are what are considered to be the significant observations on the internal structure of the nucleus at the present writing. The increased resolution resulting from thinner sections and generally improved technique have made these preliminary observations possible and more complete studies on nuclear detail and the interrelationships of nuclear components may be forthcoming in the near future.

Special Cytology of Animal Cells. The contributions of electron microscopy to special cytology—*that is, to our knowledge* of the finer structure which characterizes the cells of various tissues and organs—have been very numerous in the past few years. They have been so numerous in fact that an attempt is made here only to list the cell types involved, to make short comments on their special features, and to record the references to the articles in which further detail will be found.

Recent studies on the intercellular matrix have served to confirm earlier studies on the structure of collagen and elastin (*200*) and have demonstrated the existence of a monomeric form of collagen (tropocollagen), which is probably the precursor of periodic mature collagen in physiological fibrogenesis (*98, 99*). Interestingly enough, these studies have not brought to light anything like what might have been expected from silver impregnation and light microscopy in the way of intercellular cement. The terminal bar apparatus of light microscopy appears to be more a thickening of plasm membrane than a concentration of extracellular material (*263*).

The various types of blood cells have been examined and their specific granules described. They have been shown to possess typical mitochondria, moderate amounts of ergastoplasm, and a well developed Golgi complex (*24, 97, 136, 223*). Plasma cells have been shown to possess large amounts of highly organized ergastoplasm and a relatively hypertrophied Golgi complex (*24, 51, 52*). The formation of platelets from megakacyocytes has been described (*262*). A beginning has been made on the fine structure of peritoneal fluid cells and the characteristics of mesothelial cells from several sites have been noted (*78, 175*). The structure of endothelial cells (*179*), the lining of sinusoids of the pituitary gland (*203*), and the wall of the aorta have been analyzed (*188*).

The fine structure of cardiac and skeletal muscle has received special attention and as a result numerous papers concerned with these subjects have appeared recently. In a review of the general field of the structure of striated muscle through part of 1954 (*17*), it has been indicated that the basic structural organization of myofibrils, myofilaments, cross filaments, cross banding, sarcoplasmic reticulum, sarcosomal fine structure, and myo-tendinal junction are similar in cardiac and skeletal muscle, whether of vertebrate or arthropod. Still more recently further details of the structure of skeletal muscle with some consideration of the relationship between structure and function have been supplied (*211*). In addition, a greater number and concentration of mitochondria in red muscle than in white muscle of insects have been noted (*68*). A recent paper contains excellent detail of the fine structure of insect flight muscle, with an interpretation of this fine structure in relation to the known chemistry of muscle contraction (*121*). In vertebrates, mitochondria are more numerous in cardiac than in skeletal muscle and in some instances appear to possess tubular rather than the flattened form of cristae (*253*). It has also been noted that mitochondria are as numerous in insect flight muscle as in vertebrate cardiac muscle (*139*). One distinguishing feature of cardiac muscle, the intercalated disks, has been investigated, and it has been found that myofibrils are not continuous through it. Thus intercalated disks actually represent cell boundaries in cardiac muscle (*226, 245*).

In the study of nervous tissue considerable attention has been given to the synaptic fibers. The presynaptic fiber can be identified by the presence within it near the synapse of numerous vesicles 200 to 500 A. in diameter (*63, 64*). These vesicles and mitochondria were found to be much more numerous on the presynaptic side of the synapse than on the dendrite side and a similar concentration of vesicles was at the axon endings of neuromuscular synapses (*178*). Degeneration, first of the synaptic vesicles, followed by lysis of mitochondria was found to occur in presynaptic fibers of the ventral acoustic ganglion after destruction of the cochlea (*61*). In the crayfish giant synapse on the other hand, vesicles appeared to be concentrated on the postsynaptic side (*204*). Numerous vesicles and mitochondria are also characteristic of receptive nerve endings in association with the hair cells of the cochlea (*69*). The method of formation of the myelin sheath of vertebrates has been clearly demonstrated in embryonic material. Nerve fibers become invaginated into the accompanying Schwann cell cytoplasm, the plasma membranes of both nerve fiber and Schwann cell remaining intact. Rotation of the Schwann cell around the nerve fiber results in the building up of numerous helical layers of membranes in which the lipoproteins of the myelin sheath are laid down (*94*). A similar relationship has been demonstrated for invertebrate material (*92*) and this basic organization has been demonstrated in adult vertebrate nerve fibers (*205*). A definitive paper on the fine structure of the nerve cell body has recently appeared in which the details of the Nissl substance have been shown to be comparable in a general way to the ergastoplasm or combination of endoplasmic reticulum and Palade granules in other strongly basophilic cell types. In addition, excellent examples of the Golgi complex of neurones (called in this paper "agranular reticulum") were described. Fine fibrils 60 to 100 A. in diameter coursing through the cytoplasm of the neurones were identified as neurofilaments (*187*). A monograph on the fine structure of the thalamus has appeared (*79*).

Preliminary notes on the electron microscopy of neuroglia (*72*) of the spinal cord (*152*), cerebral cortex (*113, 156*), neurohypophysis (*67, 186*), and the organ of Corti (*230*) indicate that shortly a good deal more information on the fine structure and interrelationships of components of the nervous system will be made available.

A survey of recent observations on the fine structure characteristic of cells of certain organs reveals that valuable new information has been made available about the cells and cellular relationships in the skin, lung, stomach, intestine, pancreas, liver, kidney, and testis. A thin basement membrane at the dermoepidermal junction has been clearly visualized (*177, 218*). Bundles of tonofilaments less than 100 A. in diameter have been homologized with the Herxheimer fibers of light microscopy. Similar filaments embedded in opposing elongate granules of adjacent cells make up the intercellular bridges. Filaments do not cross the narrow space intervening between the granules (*219*). A preliminary report on the more superficial layer of stratified squamous epithelium has also appeared (*218*).

In the lung, continuity of an epithelial lining of the alveolae has been clearly established (*150*) and it has been shown that the thickness of the cytoplasm of endothelial cells of pulmonary capillaries may be as thin as 100 A. (*134*).

In parietal cells of the stomach (*214*) and principal cells of the intestine (*256*) evidence has been presented correlating fine structure with absorptive processes.

In the exocrine cells of the pancreas surprisingly little change in intracellular components was noted following injection of pilocarpine (*229*). In this cell the distinction between the ergostoplasm and Golgi complex is particularly clear (*48*). Further studies on the relationship between secretory activity and modification of fine structure in this cell type are clearly indicated.

A definitive paper on the fine structure of hepatic cells (*75*) showed that as a result of feeding after a fast, new membranes of the ergastoplasm appear first near the periphery of the cell, the small granules, probably derived from the nucleus, being added later. The mortise-tenon-like interdigitation of the lateral borders of hepatic cells and the villiform processes of the cell surface facing the sinusoid were also described and evidence for an incomplete sinusoidal lining was presented.

In the kidney the glomerulus has received considerable attention primarily in regard to its normal structure (*14, 108, 171, 189, 201, 264*) but also in regard to pathologic changes (*224*). The concept of the basic organization of the glomerulus (*174*) has not been greatly modified by these more recent studies. Higher resolution has resulted in a clearer understanding of the structure of the basement membrane (*201, 264*) and it has been suggested that the perforations of the endothelial lining are not true openings but are covered by a very thin film of endothelial cytoplasm (*264*). High resolution electron micrographs have given us further information concerning the fine structure of the proximal tubule cell under normal and experimental conditions (*202*) and of the cells of the thin segment, distal, convoluted tubule, and collecting tubule (*189*). An interesting observation, aside from vertebrate kidney cell structure, is the peculiar orientation of mitochondria in cells of the Malpighian tubule of the grasshopper (*16*). The groundwork has thus been laid for further experimental work and an understanding of pathologic changes in kidney cell ultrastructure.

As far as the testis is concerned, of primary interest is the description of the differentiation of spermatids in the cat (*36*). In this study the intimate relationship of the Golgi complex to the formation of the acrosome is demonstrated as well as the details of structure and relationships of the centriols and mitochondria to the development of the tail filament and sheath.

While still further work of a purely descriptive nature is necessary in many areas, it is clear that the basis has been laid in many other areas for experimental analysis. Descriptive studies of high quality have made it possible to predict that an increasingly high proportion of the publications in the general area of electron microscopy of tissue sections will involve the experimental approach. They have also made possible a comparison between normal and malignant cells, the beginning of which has already been made (*41, 50, 127, 128, 220*), with some assurance that any quantitative or qualitative differences uncovered would be valid.

ACKNOWLEDGMENT

Many contributors to this field were generous and helpful in making available reprints, abstracts, and references to their papers and talks. Their cooperation is sincerely appreciated.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Mass Spectrometry

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REPORTS and published researches covering the field of mass spectrometry continue to increase in scope and quantity. The nearly complete coverage of the field attempted in the previous review (*61*) is discontinued because of space limitations. Cited references appearing in the interim through November 1955 are selected as representative examples of the diverse applications. Additional references, usually for particular applications, will be found in the several reviews and surveys cited below.

Robertson (*227*) has published a short monograph giving a general introduction to the subject. A handbook on basic principles and procedures in mass spectroscopy has been prepared by Inghram and Hayden (*126*). Several books on general topics include chapters by Hipple (*114*), with emphasis on combustion studies; by Young (*284*), on hydrocarbon analysis; and by Wasserburg (*273*), on isotope dilution techniques. A general literature review was prepared by Wachter and Sachs (*264*).

A number of surveys of particular topics include: stable isotope dilution methods as analytical tools (*123*), ionization and dissociation of complex molecules by electron impact (*51, 184*), application to chemical problems (*71*), the determination of isotopic masses and abundances (*69, 189, 190*), mass spectroscopic data and nuclear binding energies (*169, 170*), and physical chemistry of isotopic differences (*54*).

Published proceedings have appeared for a conference on nuclear processes in geologic settings (*218*) held at Williams Bay, Wis., September 1953, and the conference organized by the Mass Spectrometry Panel of the Institute of Petroleum (*127*) and held in London, October 1953.

GENERAL THEORY AND ION OPTICS

Vauthier describes applications of electron optics in correction for aberration (*261*), and studies of electron beams in ion sources (*260*). The principle of the electrical mass filter suggested by Paul and Steinwadel is explained and experimentally verified (*205*). Inhomogeneous magnetic fields have been employed to increase resolving power (*2*) and for precise mass measurements (*67*). Peterlin (*209*) shows mathematically the improvement in resolution of a three-grid Bennett mass spectrometer by changing the shape of the high frequency signal. Durand (*72*) discusses line width and ion curvature.

Berry (*17*) gives the equations of motion for ions in an idealized model of the Omegatron and Edwards (*73*) describes the effects of changing tube parameters on resolution, sensitivity, and resonant frequency for the N_2^+ peak recorded by the Omegatron.

Brubaker (*30*) discusses the influence of space charge on potential distribution in ion sources. Hintenberger, Wende, and König (*113*) describe second-order, direction-focusing instruments with rectilinear image curves.

INSTRUMENTATION AND TECHNIQUES

Barnard (*10*) describes the construction and use of an all-metal, 60°, precision-engineered instrument for studies of ion sources. Ewald (*76*) describes design and techniques for an instrument with a mass resolution of 1 in 50,000. Instruments designed for solids analysis are described by Hannay (*105*), Stevans and Inghram (*246*), and Van Sickle (*259*). Reynolds (*223*) gives a complete description of an instrument for the isotopic analysis of hydrogen with low deuterium content. Other instruments designed for isotope analysis are described by Higatsberger (*112*), Krishnamurthy and Asundi (*145*), and White and Collins (*281*).

A number of papers deal with time-of-flight and ion resonance instruments. Katzenstein and Friedland (*137*) describe a time-of-flight instrument using a pulsed electron beam and the retarding-potential-difference electron gun. A radio-frequency linear decelerator mass spectrometer, described by Donner (*66*), has a mass resolution of about 100 using 24-radio-frequency gaps. Dekleva and Peterlin (*59*) demonstrate the improvement in resolving power with change in signal shape for a radio-frequency instrument. The performance of a five-stage instrument is described by Wherry and Karasek (*279*). Kramer and Le Poole (*144*) report a novel instrument in which a hollow cone of ions is formed by rotating electric fields. The mass spectrum consists of concentric circles. Ion resonance and conventional instruments have been applied to process and pilot plant control (*148, 181, 267*) and used as leak detectors (*92, 94*).

Instruments are also described with high resolution and variable band-width-measuring circuits (*206*), a rapid scanning and cathode-ray-tube display permitting a 40-peak spectrum to be scanned in 5 milliseconds (*149*), and a peak-height comparison method using alternate scanning of peaks (*249*).

A number of specific techniques are reported. White and Collins (*280*) describe positive ion counting for ion currents of 10^{-14} to 10^{-19} ampere. The characteristics of an electron multiplier in detecting positive ions are reported by Barnett, Evans, and Stier (*12*). A stabilized current supply for surface ionization sources (*1*), a trap-current-controlled emission regulator (*99*), an electron current and energy regulator (*122*), and an automatic ionization efficiency digital recorder (*183*) are described. Other papers report the use of a retarding slit in collector systems (*55*), a modulated magnetic field for mass doublet measurements (*96*), a bismuth wire fluxmeter (*186*), a vibrating-coil magnetometer (*35*), a slow, linear magnetic sweep circuit (*160*), and a trap for attenuating mercury vapors (*257*) and a means of opening sealed vacuum tubes (*191*).

Caldecourt (*34*) describes a sample inlet system useful to 250° C. that permits loading of weighed amounts of liquids or solids. Waldron (*265*) makes use of a double-inlet method for hydrocarbon analysis. A simpler introductory system is described by Palmer (*201*). A high-vacuum filament furnace for the evolution of gases from metals is reported by Consolazio and McMahon (*50*). Roberts and Walsh (*224*) utilize a vacuum lock for a solids entry system and Brown and Skahan (*28*) employ Teflon-seated and -packed toggle valves in a system for introducing hydrocarbons.

Cameron (*37*) describes an electron bombardment ion source for solids. Nief (*188*) reports on construction and operation of a thermal-emission source. Rigid source construction to prevent warping is described by Louw and de Villiers (*158*). Several reports describe improvements in the ionization region and electron beam alignment (*74, 233*). Experimental conditions for operating the ion source of a single-field instrument are reported by Hirota and Titoku (*115*), and Gvozdanović (*102*) describes an ion source with no auxillary magnetic field.

IONIZATION AND DISSOCIATION BY ELECTRON IMPACT

The accumulation of mass spectra of a variety of molecules continues as a basis for theoretical and practical studies. Collin has studied the spectra of nitroparaffins (*46*), dialkylnitrosoamines (*47*), and other nitrogen compounds (*45*). Dibeler, Mohler, and de Hemptinne (*62*) report the spectra of all of the deuteroethylenes and point out simple mathematical relations between the extent of deuteration and the dissociation probabilities. Florin, Wall, Mohler, and Quinn (*82*) discuss the mass spectra and thermal decomposition of the cyclic trimer of dimethylphosphinoborine. Friedman (*89*) obtains evidence for monomer and dimer in the mass spectrum of lithium iodide vapor. Friedman, Irsa, and Wilkinson (*90*) report the spectra of a number of cyclopentadienyl metal compounds, correlating differences in spectra with the nature of the metal-to-ring bonding. Kerwin (*139*) observes clusters of up to eight phosphorus atoms in vapor-phase studies of red phosphorus. Searcy, Freeman, and Michel (*236*) report the composition of silver vapor to be: $Ag^+:Ag_2^+:Ag_3^+:Ag_4^+ = 15:7:30:1$ with a possibility of some heavier species. Lane, Katzenstein, and Friedland (*146*) have observed that characteristic spectra of steroids can be obtained from pyrolysis products. Studies are also reported for methane at elevated temperatures (*143*), octaborane (*238*), *s*-triazine (*134*), organochlorosilanes (*244*), and "split" peaks in the mass spectra of hydrogen (*258*).

Molecular dissociation patterns induced by electron impact are discussed by Thompson (*252*). Momigny describes the behavior of halogen-substituted benzene rings (*177*) and the influence of ionizing electron energy on mass spectra (*178*). Pahl (*199, 200*) finds empirical correlations in the amount of fragmentation observed in the mass spectra of a number of organic molecules including linear alcohols, mercaptans, and alkyl benzenes. Rosenstock, Wahrhaftig, and Eyring (*229*) discuss the nature and origin of metastable ions in terms of a general theory of mass spectra. Schaeffer (*232*) and Schaeffer and Owen (*234*) discuss the effect of isotopic substitution on the mass spectra of simple molecules. Aspects of the ionization and dissociation of molecules in terms of molecular orbital theory (*163*) and of group theory adiabatic correlations (*164*) are discussed by McDowell. He also gives (*162*) a general discussion of the nature and origin of mass spectra of a number of simple molecules. Mohler, Dibeler, and Reese (*176*) demonstrate that a process of formation of methyl ions with excess kinetic energy is common to a variety of molecules.

Studies of radical formation (*154, 155*) include the ionization potentials of ethyl, isopropyl, and propargyl radicals (*78*), the HO_2 radical (*84*), OH, HO_2, and H_2O_2 production (*85*), the ionization potentials of methyl, allyl, and benzyl radicals (*156, 157*), the ionization and dissociation of methyl radicals (*266*) and of methylene, methyl, and methane (*147*), and the detection of radicals in mercury-photosensitized decomposition of acetone (*79*).

Robertson (*225*) reports on investigations of the HO_2 radical and of an unsuccessful search (*226*) for H_2O_4 and HO_4 in gaseous mixtures containing HO_2 and O_2 Tsuchiya (*256*) detects the free OH radical in the thermal decomposition products of water vapor.

Fox and coworkers extend their studies on ionization processes using essentially monoenergetic electron beams. They report probability curves near threshold for carbon monoxide, nitrogen, propylene, and benzene (*86*), for singly charged helium and doubly charged neon, argon, and krypton (*111*), and for zinc, cadmium, and mercury (*109*). Frost and McDowell (*91*) observe the excited states of the N_2^+ ion using apparatus similar to that of Fox and coworkers. Clarke (*41*) using an electrostatic electron filter to obtain a nearly monoenergetic electron beam, gives results of studies on N_2^+, N^+, and Xe^{++} ions. Ionization and appearance potential studies have also been reported for methyl and ethyl fluoride (*165*), methyl and methylene bromides and chlorides (*100*), dimethylamine (*25*), benzene, toluene, pyridine, cyclooctatetraene, pyrrole, furan, thiophene (*193, 194*), and substituted acetylenes (*44, 87*). Thorburn (*253*) gives new data on the appearance potentials of O^+ and O^- ions and Hagstrum (*104*) gives a reinterpretation of previous electron impact experiments in carbon monoxide, nitrogen, nitric oxide and oxygen. Kandel (*135, 136*) reports appearance potential and kinetic energy studies of a number of simple molecules. Morrison (*182*) reports on the electron spectra of some simple molecules. Finkelnburg and Humbach (*81*) discuss ionization energies of atoms and atomic ions. Field and Franklin (*80*) review the implications regarding certain aromatic reactions from electron impact studies. Schissler and Stevenson (*235*) compute the benzyl-hydrogen bond dissociation energy, whereas Farmer, Henderson, McDowell, and Lossing (*77*) conclude that a reliable calculation of the value cannot be made by a direct method.

Studies of negative ions include those formed from sulfur dioxide (*185*), carbon tetrachloride and titanium tetrachloride (*168*), sulfur hexafluoride (*110*), hydrogen chloride and bromide (*101*), the monohalomethanes (*63*), and hydrocarbon gases (*8*). Donnally and Carr (*65*) observe seven transitions for negative metastable ions with half lives of the same order as positive metastable ions. Marriott and Craggs (*166*) report studies of structure and of positive and negative ion formation in complex polyatomic molecules. They also describe (*167*) details of construction and use of a Lozier apparatus for studies of collision processes of various negative ions with molecular gases. Rosenbaum and Neuert (*228*) give appearance potentials for negative ions of a number of simple polyatomic molecules and also correct a previous report in which HS^- and HSe^- ions were incorrectly identified as S^- and Se^- ions; thus leading to erroneous conclusions concerning the electron affinities of the sulfur and selenium atoms.

APPLICATIONS TO PHYSICAL CHEMISTRY AND CHEMICAL PHYSICS

The mass spectrometer is particularly applicable to studies of reaction products and processes and a number of papers describe vapor-phase photolysis reactions of methyl ethyl ketone (*5*), acetic acid (*6*), acetone (*231*), hexafluoroacetone (*7*), and cyclooctatetraene (*248*). Boivin and MacDonald (*20*) have studied the pyrolysis of ethyl mercaptan. Danby and others (*57*) discuss modes of decomposition of *n*-pentane. Flynn and Hulbert (*83*) report on the reduction of ethylene platinous chloride with deuterium. Momigny (*179*) describes a study of the absorption of phenol on nickel catalysts.

Stevenson and Schissler (*247*) report on the nature of reactions $X^+ + YH = XH^+ + Y$ in the mass spectrometer. Ogg and Sutphen (*192*) describe the effect of added ozone on the exchange reaction, $O^{16}O^{16} + O^{18}O^{18} \rightleftarrows 2\ O^{16}O^{18}$. Osberghaus and Taubert (*197*) study wall reactions of organic molecules at high temperatures. Prescott, Hudson, Foner, and Avery (*217*) used a quartz sampling probe immersed in flame to study composition profiles in premixed laminar flames. Diffusion coefficients of gases in solids are reported by Gentner and Trendelenburg (*95*).

A radio-frequency probe (*23*) is used to sample ion concentrations in a discharge tube. Beaussier (*15*) studies chemical reactions initiated by gas discharge. Becky and Dreeskamp (*16*) report relative ion abundance in the discharge plasma. Thorburn (*254*) describes preliminary studies of the nature of permanent dissociation of sulfur hexafluoride in corona discharge.

Chupka and Inghram (*39, 40*) report a direct determination of the heat of sublimation of carbon using a tantalum Knudsen cell with a graphite lining. As found by Honig (*119*), the vapor consists of various species (C_1, C_2, C_3, etc.) probably up to C_8.

Chupka and Inghram conclude that the heat of sublimation of C_1 is 170 kcal. Honig (*120*) has also studied the heats of sublimation and evaporation of germanium and silicon. Köhl (*142*) also reports on the composition of the vapor phase of germanium, tin, and silicon. Johnson, Hudson, and Spedding (*130*) describe measurements of latent heats of metals. Inghram and coworkers also report on studies of metal–metal oxide systems using a Knudsen cell. These include $Si-SiO_2$ (*213*), $Al-Al_2O_3$ (*214*), strontium oxide and magnesium oxide (*212*), and barium oxide (*124*). Other reports include studies of the alkaline earth oxides (*207*), strontium oxide on platinum (*18*), and negative ion emission from oxide cathodes (*98*, *262*). Burns (*31*, *32*), using essentially monoenergetic electrons obtained by the Fox method, determines 225 kcal. for the heat of dissociation of nitrogen. Lindholm (*151*), using a difficult technique of fragmentation of nitrogen by bombardment with atomic ions, concludes the lower value of 170 kcal. is correct. Sheenan (*239*) proposes a method for determining $D(N_2)$ by investigating the mass spectra of N_2^{15} and N_2^{14} and their approach to equilibrium. Lindholm (*150*, *152*, *153*) describes further experiments on ionization and dissociation of molecules by collision with atomic ions. These can provide direct experimental evidence as to energy states of ions, a very important uncertainty in electron impact studies. Henglein (*108*) utilizes the unique properties of a parabola spectrograph to obtain information on dissociation processes from secondary ion formation. Potter (*215*) gives cross sections for charge transfer collisions of low-energy ions in nitrogen and oxygen. Hasted (*107*) reports detachment cross sections for collisions of negative atom ions of oxygen, chlorine, and iodine in the rare gases. Smith and Branscomb (*242*) report details of a photo detachment experiment that gives a new value for the electron affinity of oxygen atoms (1.48 e.v.) and demonstrates the absence of the supposed excited state of the O^- ion (at 2.2 e.v.). Moore and Hughes (*180*) describe experiments of electron bombardment of solid compounds to determine heats of formation. Inghram and Gomer (*97*, *125*) report on the application of the field microscope to mass spectrometry and suggest its use in the study of fragmentation processes on catalyst surfaces, detection of molecular beams, and measurement of decay times.

ANALYTICAL APPLICATIONS

A comparison of principles, methods, and results of mass spectrometric, infrared, ultraviolet, and Raman spectroscopy and some distillation procedures is presented by Powell and Ross (*216*). Volk (*263*) gives a statistical analysis of accumulated data on a carbureted water gas, indicating the different components of variance.

Hannay (*106*) describes analytical techniques for a variety of metals and semiconductor materials. Palmer and Aitken (*202*) discuss the problem of purity analysis with particular reference to isotopic analysis of solids. Tilton, Aldrich, and Inghram (*255*) describe the determination of thorium in minerals of igneous rocks by the use of isotope dilution techniques.

Analytical methods for micro quantities of gas are given in two papers (*243*, *272*). Automobile exhaust gas is analysed by Walker and O'Hara (*268*). Quiram, Metro, and Lewis (*220*) describe the determination of hydrocarbon gases as air contaminants. Johnson and Meadows (*129*) report preliminary results of the first investigation of ambient positive ion composition to 219 km. by rocket-borne mass spectrometer. Bartels and others (*14*) measure respiratory dead space by single breath analysis. Brown and others (*27*) determine hydrocarbons in hydrogen by a palladium tube. Sendroy and others (*237*) report identification and quantitative estimation of iron pentacarbonyl in commercial carbon monoxide. Kirshenbaum and Grosse (*140*) extend the isotopic tracer technique for determining 0.01 to 0.1 weight % oxygen in copper. Hageman and Katwijk (*103*) give the experimental conditions for satisfactory analysis of several alcohol-water mixtures and of mixtures of nine carboxylic acids. Bond (*21*) discusses the analysis of deuterated hydrocarbons. Freeman and Serfass (*88*) utilize a technique of relative abundance ratios by direct comparison of peak heights.

Quayle (*219*) reports a study of mass spectrometric methods of analysis of hydrocarbons in the gasoline range. Several papers describe analytical methods for lubricating and heavy petroleum gas oils (*161*, *174*, *195*), for saturated hydrocarbons of high molecular weight (*43*), alcohols (*29*), and other organic molecules (*141*). Bradt and Mohler (*24*) report the analysis of fluorinated polyphenyls containing as many as eleven phenyl groups.

A number of papers describe automatic methods of varying complexities. Continuous sampling of gas streams is described by Mattraw, Patterson, and Pachucki (*172*). Jones (*131*) discusses nonelectronic computing methods, and other papers describe the application of IBM equipment (*13*), digital computing methods (*11*), and electronic computers (*56*). Dudenbostel and Priestley (*70*) report on completely automatic equipment utilizing peak selectors, an analog-to-digital converter, and a high speed computer capable of providing 30 gas analyses, each with 20 components, per 8-hour shift.

PRECISE MASS MEASUREMENT AND ISOTOPE ABUNDANCE

Accumulated mass spectrometric data on atomic masses coupled with nuclear disintegration energies permit the computation of isotopic masses and binding energies of all known nuclei for $A < 34$ (*270*), $33 < A < 202$ (*271*), and $A > 201$ (*121*). Mattauch and Bieri (*171*) report a new determination of the masses of H^1, D^2, He^4, C^{12}, and N^{14}. Duckworth and coworkers describe studies of nuclear shell structure in the rare earth mass range (*116*), the reliability of atomic masses in the chromium-germanium region (*138*), the atomic masses of ruthenium-98, ruthenium-99, osmium-189, and osmium-192, (*208*), and atomic weights of the monoisotopic elements (*68*). Collins, Johnson, and Nier (*48*) report atomic masses in the region between gallium-69 and niobium-93. Delfosse (*60*) uses sensitive nuclear plates to determine precise measurements of radioactive isotope masses.

Mohler (*175*) has announced the establishment of a bank of elements and compounds available as isotope references samples. Clarke, Denton, and Reynolds (*42*) report the determination of the absolute concentration of deuterium in Thames river water. The analysis of hydrogen-deuterium mixtures is described by Newton and Mohler (*187*), of heavy water by Roth (*230*), and of hydrogen-tritium mixtures by Jones (*133*). Cameron (*36*) reports variations in the natural abundance of the lithium isotopes. Collins and Rourke (*49*) comment on the use of surface ionization sources in the analysis of lithium isotopes. Craig reports carbon-13 variations in Sequoia rings and the atmosphere and the concentration of carbon-13 in plants and the relationship between carbon-13 and carbon-14 variations in nature (*53*). Dole, Lane, Rudd, and Zaukelies (*64*) compare the isotopic composition of atomospheric oxygen and nitrogen for samples collected at locations all over the world. Holt and Hughes (*118*) describe a preparation of nitrogen samples for isotopic analysis. Ory, Prescott, and Lyman (*196*) discuss the interference of nitric oxide in nitrogen isotope analyses. Brodskiĭ and others (*26*) describe a rapid micromethod for isotopic determination of oxygen in water. Dansgaard (*58*) reports O^{18} abundance in 109 samples of fresh water from various parts of the world. Owen and Schaeffer (*198*) report no observable differences in the natural abundance of chlorine isotopes. Cameron (*38*) concludes that no significant variation in bromine isotope abundance occurs in nature. Hogg (*117*) concludes from observations on titanium tetrachloride that natural variations of titanium must be less than 1%. White, Collins, and Rourke (*282*) report the discovery of a rare isotope of tantalum. Geiss (*93*) reports analyses of "common lead." Ehrenberg and Horlitz (*75*) tabulate isotope distributions in lead ores obtained mainly from West Germany. Buttlar (*33*) discusses the advantages and disadvantages in the use of RaD as a dilution isotope in the estimation and the isotopic analysis of lead.

NUCLEAR CHEMISTRY AND GEOCHEMISTRY

Mass spectrometry and isotope dilution techniques are used to obtain absolute fission yields in a study of the fine structure of uranium-233 fission (*245*). Petruska and coworkers (*211*) study the absolute yields of 28 mass chains in the thermal neutron fission of uranium-235, as well as the yields of cesium isotopes (*210*). They also report on the neutron absorption cross section of xenon-135, and the relative abundances of neodymium and samarium isotopes from uranium-235 and -238 (*173*). From the same isotopes, yields are also reported for the isotopes of xenon and kypton (*269*). Balestrini (*9*) uses isotope dilution methods to measure cross sections for the production of xenon-126, -127, and -128 from iodine-127 by deuteron irradiation. Boyd, Brown, and Lounsbury (*22*) report on the study of natural and neutron-irradiated chlorine. Half lives are determined for tritium (*132*) and for strontium-90 (*283*).

Amirkhanov, Gurvich, Shanin, and Sardarov (*4*) measure the amount of radiogenic argon in geological formations to determine absolute age. Shillibeer and Russell (*241*) also discuss radiogenic argon measurements. Wetherill (*277*) reports on the variation in isotopic abundances of neon and argon extracted from radioactive minerals. Argon-40–potassium-40 dating techniques have been described (*240, 274*) and applied to determine the age of meteorites (*276*), feldspars (*275*), and micas (*278*).

Thode and coworkers (*250, 251*) discuss the sulfur isotopes in nature and the origin of the large sulfur deposits in southwestern United States. Allenby (*3*) reports unexpectedly large variations in the isotope ratios of silicon in rocks. Boato (*19*) studies the isotopic composition of hydrogen and carbon in the carbonaceous chondrites. Rankama (*221, 222*) considers the carbon isotope abundance in ancient rocks as an indication of biogenic or nonbiogenic origin. Jeffery, Compston, Greenhalgh, and de Laeter (*128*) report on the carbon-13 abundance of limestones and coals. Craig (*52*) discusses the geochemical implications of the isotopic composition of carbon in ancient rocks. Lowenstam and Epstein (*159*) use the oxygen isotope method to measure paleotemperatures of the postaption cretaceous. Patterson (*203*) discusses the lead-207–lead-206 ages of stony meteorites. Patterson, Tilton and Inghram (*204*) give a critical analysis of this method in regard to the reliability of conclusions on the age of the earth.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Emission Spectroscopy

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THE principal advances in fundamental research and applications of atomic emission spectroscopy during the years 1945–53 were reviewed in four previous reports (*64–67*). As this period and the present is commonly called the "atomic age," it seems appropriate to review some new knowledge concerning atoms and their uses acquired via spectroscopy during the years 1954–55.

STANDARD WAVE LENGTHS

The last review (*67*) mentioned the first meeting of an International Advisory Committee for the Definition of the Meter. That committee recommended that the meter be defined by a wave length in vacuum emitted by an atom which has a spectrum free from hyperfine structure and all other perturbing influences. It also recommended that the new definition be established by letting the wave length value 6438.4696 × 10^{-10} meter of cadmium red radiation serve as an intermediary. The transition from air to vacuum must be made by the dispersion formula for normal air adopted in Rome in 1952 by the Joint Commission for Spectroscopy (*67*).

In previous reviews (*65–67*) it was mentioned that artificial mercury-198, made by transmuting gold-197, had supplied a finer standard of length than the red radiation of natural cadmium. A preliminary value of 5462.270240 ± 0.000008 × 10^{-10} meter for the unperturbed vacuum wave length of green radiation from mercury-198 has been reported by Kessler and Meggers (*48*). This was measured with Fabry-Perot interferometers relative to a vacuum value of 6440.24907 × 10^{-10} meter assumed for the red radiation of cadmium. Since the wave length of cadmium red radiation is more exactly definable than any ruled meter, and the vacuum wave lengths of mercury-198 radiations can be more precisely specified than either, it seems justifiable to add arbitrarily two decimal places.

Having intimated that the most precise unit of length will be found in atomic spectra, it should be mentioned that the most precise unit of time will also be found in atomic spectra (*25*). "A frequency standard based on a natural resonant frequency of the cesium atom has been constructed at the National Physical Laboratory and used for calibrating the quartz clock standards with an accuracy of ± 1 part in 10^9 (0.0001 second per day). The resonance observed is that due to transitions between the hyperfine structure energy levels caused by the interaction between the nuclear and the electron spins. This appears to be the first example of the use of an atomic frequency standard with an accuracy greater than that obtained by using a unit of astronomical time. This also represents the highest accuracy ever achieved in the measurement of any physical quantity in terms of a definitive standard."

The wave lengths of all spectral lines (except the primary standard) are measured relative to secondary (or tertiary) standards. It is perhaps not generally appreciated that the spectacular progress in the description, interpretation, and application of atomic spectra during the past three decades can be credited, at least in large part, to the existence of an international system of secondary standards of wave length derived from a direct current arc between iron electrodes. This was not discussed in previous reviews (*64–67*) because there was nothing new to report about iron standards from 1945 to 1953. However, significant progress with international secondary standards was made in 1955, and because it is exactly a half century since they were projected, it may be of interest to review, briefly, the history of this international scientific cooperation.

In 1905 the International Union for Cooperation in Solar Research proposed that a primary standard of wave length (and definition of the angstrom) be adopted, and that secondary standards at 50-A. intervals in the arc spectrum of iron be determined by an interference method devised by Fabry and Perot in 1902. In 1907 the Union adopted as the primary standard 6438.4696 A., the wave length of red radiation from cadmium measured relative to the meter by Benoit, Fabry, and Perot. In 1910 the Union met in Pasadena, and adopted 42 secondary standards in the arc spectrum of iron ranging from 4282.408 to 6494.993 A., the values being the means of three independent determinations, one from France, one from Germany, and one from the United States.

After World War I, responsibility for secondary standards of wave length was assumed by the International Astronomical Union which in 1922 adopted 80 iron wave lengths, 3370.789 to 6750.163 A. In 1922 the arc gap was increased from 6 to 12 mm., and when the iron secondary standards were remeasured they had slightly smaller values. In 1928 values were adopted for 235 iron standards ranging from 3370.787 to 6677.993 A., and by 1935 the number had increased to 306 and the range to 2447.708 A. This international system of secondary standards of iron wave lengths has been the foundation of modern spectroscopic measurements up to the present time.

Individual values of iron wave lengths from 2100.794 to 10216.351 A. had been reported, but confirmatory observations were lacking, so the number of official standards could not be increased. In recent years additional measurements were made, so that by 1954 the means of three or more independent, concordant determinations existed for 575 iron wave lengths ranging from 2501.133 to 9372.904 A. Instead of adopting these means as secondary standards of wave length, a new procedure was followed by the International Astronomical Union—the best relative atomic energy levels were derived from these means, and then new secondary standards were calculated from permitted combinations of the energy levels. Assuming that the combination principle is exact, this procedure reduces residual random errors of the original means and permits the calculation of additional wave lengths with equal precision. Thus, instead of adopting seven-figure observed values of 575 iron lines with wave lengths from 2501.133 to 9372.904 A., the International Astronomical Union adopted (*92*) eight-figure calculated values of 1016 iron wave lengths ranging from 2084.1218 to 11973.067 A. These new secondary standards of wave length supersede all previous iron standards; they climax a half century of effort in this direction, and it is now recognized that further improvements in standard wave lengths must be sought in other spectra.

The maximum accuracy with which relative wave lengths can be measured is limited by line widths resulting usually from the Doppler effect and sometimes collision broadening. This is easily seen in the Fabry-Perot method of comparing wave lengths. The parallel-plate interferometer is illuminated simultaneously by a cadmium lamp and an iron arc, and the interference fringes are projected on the slit to a stigmatic spectrograph. Then the double distance, $2d$, between the interferometer plates can be expressed as the product of any wave length, λ, and its interference order, n (total retardation), at the center of the pattern. Thus, $2d = \lambda_{\text{Cd}} \times n_{\text{Cd}} = \lambda_{\text{Fe}} \times n_{\text{Fe}}$, and $\lambda_{\text{Fe}} = \lambda_{\text{Cd}} \times n_{\text{Cd}}/n_{\text{Fe}}$. In general, the order number, n, consists of an integer and a fraction. The integers can be determined without error, so that the accuracy of relative wave-length determinations depends solely on the errors in fractional orders obtained from measurements of the interference patterns. These errors are usually about 1/100 of an order (or wave) when good fringes are available for measurement. Now the maximum order (consistent with good fringes) for lines emitted by an iron arc at atmospheric pressure is about 50,000. Therefore, if the fractional orders have 1% error, the relative wave lengths of these iron lines cannot be determined closer than 1 part in 5,000,000, and individual secondary standards cannot be measured more accurately even though they have been calculated to eight figures (*92*).

The present iron standards cannot be measured with high accuracy because they are too wide. Their width is due mainly to Doppler effect because of very high temperature. Indeed, a temperature of 6300° K. ± 10%, has been deduced (*13*) from the Doppler width of iron lines emitted by a 5-ampere direct current arc. Furthermore, collision broadening is present because the arc is operated at atmospheric pressure.

The only practical way of improving the iron standards is to replace the high-temperature arc in air by sources operating at lower temperatures and much lower pressures. A hollow iron-cathode lamp containing neon at 3 mm. of mercury pressure has been proposed (*22*) as a superior source of iron standards, and some preliminary wave lengths from this source have been reported (*87*). One defect of this lamp is that its intensity is only a fraction (1/100) that of the international iron arc. An alternative brighter source is an electrodeless iron-halide lamp containing argon at 2 mm. of mercury pressure and excited with microwaves. Such lamps have been tested with interferometers and compared (*88*) with the hollow iron-cathode lamp and with the international iron arc. The operating temperature of either the iron cathode or the electrodeless lamps is near 800° K., and since the total gas and vapor pressures are only a few millimeters of mercury, the iron lines are much narrower than those from the international iron arc. In fact, with either of these low temperature-pressure sources good interference fringes are observed with interference orders three or four times the maximum allowed by the iron arc, so that iron wave lengths can be measured with an accuracy of 1 part in 15,000,000 or 20,000,000 if required.

However, even this increase in accuracy of secondary iron standards will not meet future requirements. Iron is a light atom (mass 56) compared with cerium (mass 140) or with uranium (mass 238). In comparable light sources, the width of iron lines will be double the width of uranium lines. How can the wave lengths of uranium lines be measured accurately with inferior standards? With only slight exaggeration one could pose a similar question; how can a meter be measured accurately with a piece of string?

Furthermore, iron has a simple spectrum compared with those characteristic of rare earth metals, and even the present list of 1016 computed secondary standards has several gaps of about 100 A. and one of nearly 1000 A. How can the extremely rich spectra of rare earths be accurately described with poorer standards and large gaps? Indeed, the major spectroscopic task of the future is to describe these very complex spectra with sufficient accuracy to find atomic energy levels, excitation potentials, and electron configurations. In order to distinguish true physical regularities from fortuitous facts in these complex spectra it is desirable to determine relative wave lengths with the greatest accuracy attainable. For this purpose new secondary standards must be measured from 2000 to 12000 A. at intervals of about 5 A. These standards must be obtained from practical sources that emit thousands of sharp lines of comparable intensity. These specifications are fortunately met by metal-halide lamps of thorium or uranium excited by microwaves. Thorium and uranium are Nature's purest and heaviest metals. Thorium is 100% mass 232, and uranium is 99.3% mass 238. Since both masses are single and even, the spectra are free from isotope shifts and from hyperfine structure. Both metals form volatile halide compounds which are used in making electrodeless lamps (*21*). Preliminary tests of such lamps with interferometers indicate that relative wave lengths of thorium can be measured with orders of interference of 400,000 or more, and a resolving power of 700,000 has been demonstrated (*95*) with uranium lamps. Thus, there are actually good prospects that secondary standards 10 times more accurate than those observed in the iron arc can be derived without gaps in the electrodeless-lamp spectra of either thorium or uranium. Until such standards are measured (relative to mercury-198 standards) spectroscopists will continue to use the present inferior iron standards (*92*).

A few standards of better quality are now available from neon, argon, or krypton excited in Geissler tubes. During its ninth general assembly the International Astronomical Union adopted (*92*) 30 neon wave-length standards (5400.5617 to 8082.4581 A.), 56 argon standards (3947.5047 to 9784.5020 A.), and 31 krypton standards (4273.9703 to 6456.2910 A.). These standards, however, do not extend into the ultraviolet and are too irregularly spaced and widely separated to be useful in the measurement of rich rare earth spectra.

Another urgent need in spectroscopy is a system of true standard wave lengths in the vacuum ultraviolet that is shorter than 2000 A. No interferometer measurements of wave lengths have ever been made in that region, and the best wave lengths have been either measured in overlapping orders of grating spectra or calculated from spectral terms evaluated from measurements in the longer wave regions. The present tendency is to compute extreme ultraviolet wave-length standards from spectral terms. Thus, the wave lengths of 23 C III lines (291.3261 to 574.2809 A.) suitable as standards have been calculated (*7*), and computed values of 35 Mg II wave lengths (884.6967 to 1753.4744 A.) have been proposed (*79*) as standards. Likewise, interferometric measurements of germanium lines have led to the calculation (*69*) of 22 standard lines in the vacuum ultraviolet between 1998 and 1691 A. A more extensive and useful list of provisional wave-length standards in the vacuum ultraviolet has been published by Wilkinson (*98*), who measured the wave lengths of 245 atomic emission lines (875.092 to 1942.273 A.) mainly from carbon, nitrogen, and oxygen, commonly occurring as impurities in conventional light sources. These lines were measured in the first order of a 21-foot vacuum grating spectrograph relative to Cu II, Fe I, and Fe II, whose wave lengths can be calculated accurately from spectral terms. This work increases the number of previously known vacuum ultraviolet provisional standards and places them on a consistent wave length scale with an average internal error of ±0.003 A. and an absolute error of less than ±0.005 A.

SPECTRAL LINE INTENSITIES

For many years spectral line intensities were represented by numbers arbitrarily assigned by individual observers. In 1925, when the quantum theory of atomic spectra was developed, it became possible to calculate the relative intensities of lines in multiplets, and photographic photometry was applied to the quantitative measurement of such intensities. However, after the sum rules had been tested, such measurements were discontinued in fundamental spectroscopy but adopted in applied spectroscopy for making quantitative chemical analyses by measuring the intensity ratios of internal-standard and analysis lines. Consequently, at the present time the wave lengths of several hundred thousand atomic radiations are known to six or seven figures, whereas the relative energies of only a few hundred lines are known to two or three figures and the rest are still represented by arbitrary numbers.

Some reports of calculated or observed intensities published since 1945 were mentioned in previous reviews (*64–67*); to these the following may be added. Relative gf values for 256 lines (2987 to 6450 A.) of Co I were obtained (*52*) from total absorptions of faint lines appearing when cobalt was vaporized in an electric furnace combined with sources of a continuous spectrum. Relative oscillator strengths of 80 lines (3800 to 5300 A.) in Fe I were measured (*1*) in emission from an electric furnace at 2800° K. Relative intensities of 478 Ni I lines (3359 to 5893 A.) emitted by a 2-ampere arc between nickel electrodes were recorded (*40*) photoelectrically by means of a ratio recorder which reported the ratio of the output of a scanning tube to that of a fixed tube placed on a control line, thus minimizing the influence of arc fluctuations. An accuracy of better than 1% is claimed for the good lines.

The above-mentioned observations, as well as practically all preceding ones, were calibrated with tungsten lamps operated at a measured temperature to provide a theoretical energy distribution. As it is impractical to use such lamps in the extreme ultraviolet, there are at present no reliable measurements of spectral intensities below about 3000 A.

One of the greatest needs in precise spectroscopy today is an intense, steady light source with reproducible and known distribution of energy in the extreme ultraviolet.

A new method of measuring atomic transitions has been proposed (*11*); it requires a steady source and two photomultipliers. By observing coincidences between photons emitted in a cascade transition and detected by the two photomultipliers, the behavior of individual atoms is observed. This direct application of nuclear coincidence techniques allows one to measure absolute source strengths, relative transition probabilities, and lifetimes of intermediate states. By this method the lifetime of the $7s\,^3S_1$ state of mercury has been found to be $(1.12 \pm 0.02) \times 10^{-8}$ second. It remains to be seen whether this method of measuring intensities is applicable to complex atomic spectra.

Theoretical calculations of intermediate coupling line strengths have been compared (*32*) with observed intensities of $3s - 3p$ and $3p - 3d$ transitions in O II and in Ne III, and of $4s - 4p$ transitions in S II and A II. The calculation of oscillator strengths, including configuration interaction, for transitions between $2p^33p$ and $2p^33s$ in O I has been published (*53*).

ZEEMAN EFFECT

After the "anomalous" Zeeman effect was explained in 1925 it became the most effective aid in the quantum interpretation of atomic spectra. Resolved and measured Zeeman patterns yield uniquely for both atomic energy levels the atom's total momentum, j (related to the number of magnetic components), and the orbital angular momentum, l (associated with the magnetic splitting factor). The Zeeman effect was extensively exploited in the interpretation of atomic spectra, and this program would probably have been completed by now if it had not been interrupted by World War II. The Zeeman effect of gold recently reported by Green and Maxwell (*35*) probably presents the last of the prewar observations; these results confirm some earlier analyses of Au I but indicate that published interpretations of Au II will require critical review.

New data on the Zeeman effect in the Mo II spectrum have been indicated by Kiess (*51*), in the Hf spectrum by Meggers (*68*) and in the Os I and Os II spectra by van den Bosch (*8*). By employing electrodeless metal-vapor lamps and an Echelle-Littrow spectrograph, superior Zeeman spectrograms have been obtained for plutonium (*37*) and for zinc, cadmium, mercury, iron, tin, and tellurium (*38*).

In O I small departures of accurately measured magnetic splitting factors from values given by simple vector-coupling theory are explained (*45*) by an improved theory of the Zeeman effect of atomic oxygen.

TERM ANALYSIS

The critical compilation and publication of "Atomic Energy Levels," mentioned in previous reviews (*65–67*), by calling attention to lacunae or defects, continues to provide a strong incentive for the term analysis of atomic spectra. This project was begun in 1946 with the object of publishing at intervals of about 3 years a series of volumes containing all available information about atomic energy levels derived from the analysis of optical spectra. Such information includes relative energy levels, spectral terms, quantum numbers, magnetic splitting factors, electron configurations, and ionization potentials.

Volume I was published in 1949, and Volume II in 1952. Volume III of this series is now in press (*72*); it will present all available atomic data for 34 elements (42Mo to 57La, and 72Hf to 89Ac). This volume will contain much new material especially for spectra of molybdenum, technetium, ruthenium, rhodium, iodine, hafnium, tantalum, tungsten, rhenium, osmium, iridium, polonium, and actinium. Intimations that have appeared in scientific publications during the last biennium are as follows: Mo II (*51*), Mo III (*78*), Tc I (*10*), Ru I (*90*), I I (*73*), Hf I (*68*), Po I (*16*).

Recent contributions that supplement or supersede the atomic information already published in Volumes I and II of "Atomic Energy Levels" have appeared in the past 2 years. Thus, a new study of C III has yielded (*7*) 121 new lines, 21 new levels, and a connection between singlet and triplet terms. The spectral series of O I have been extended (*58*). New observations of Mg II from 2790 to 11620 A. disclosed (*79*) 26 new lines and led to a revision and extension of the term system.

The former fragmentary analysis of Ni III has been greatly extended (*31, 85*), and an ionization potential of 36.16 e.v. has been derived from spectral series. Likewise, 58 new levels have been found (*44*) for Nb III and a spectroscopic ionization potential of 27.5 e.v. was calculated.

Infrared lines of iodine, detected (*24*) with a lead sulfide cell, indicated several new levels. Infrared photography of yttrium (*9*), palladium, and platinum spectra (*50*) recorded some new lines but revealed no new energy levels. For purposes of term analysis as well as spectrochemical identification entirely new descriptions of the arc and spark spectra of ruthenium were published (*49*). The use of electrodeless metal-vapor lamps for observing spectra in regions where molecular spectra mask atomic lines has been demonstrated (*97*); 131 new Ti I lines were measured betweeen 5618.32 and 5929.27 A., a region in which a titanium arc in air shows a strong spectrum of titanium oxide.

Although the compilation of atomic data from rare earth spectra is postponed to 1956 when the preparation of the fourth volume of "Atomic Energy Levels" will begin, it is gratifying to see that researches on these spectra are in progress. Thus a notable extension of the analysis of the first spectrum of lutetium has been published (*54*). As regards the transuranic elements, progress is being made (*6, 19*) with the optical spectra of plutonium, and a preliminary term analysis of the first two spectra of americium has been reported (*28*). Progress with the description and interpretation of the successive spectra of these unstable elements is bound to be slow because too many of the world's best scientists are still engaged in developing offensive "weapons that stagger the imagination."

HYPERFINE STRUCTURE

The papers mentioned under term analysis deal only with the fine structure of atomic spectra, excluding hyperfine structure and isotope shifts arising from interactions between optical electrons and atomic nuclei. Since such interactions yield highly quantitative information about the atomic nuclei, they will continue to be investigated with the hope that they will lead to an acceptable theory of nuclear structure. The nuclear properties that are derived from spectral hyperfine structures are the mechanical moment, I, the magnetic moment, μ, and the electric quadrupole moment, Q.

The spectroscopic measurement of these nuclear moments has been under way for more than a quarter of a century, and in recent years entirely different methods, intrinsically more precise, have been applied. Despite the competition of microwave techniques and of atomic or molecular beam magnetic resonance experiments, the investigations of optical hyperfine structures continue unabated. Only a selection of the recent papers on hyperfine structure will be mentioned here. The hyperfine structure of Yb 6489 A. confirms $I = 5/2\ (h/2\ \pi)$ for 70Yb^{173}, which does not fit the shell model of nuclear structure (*56*). From the hyperfine structure of the resonance line (3694 A.) of Yb II new values of μ (Yb^{171}) = $+0.49 \pm 6$ and μ (Yb^{173}) = -0.67 ± 0.01 nuclear magnetons have been derived (*57*). New values are reported (*46*) of Q (Ta^{181}) = 4.3 barns, and Q (Lu^{175}) = 5.7 barns.

The nuclear moments of artificial or unstable isotopes are being investigated intensively. For example, krypton-85 resulting from the fission of uranium-235 has been found (*77*) to have $I = 9/2\ (h/2\pi)$, like krypton-83, but μ and Q are larger than for the stable isotope. The hyperfine structure of some Ac II lines has disclosed (*29*) for actinium-227 a value of $I = 3/2\ (h/2\pi)$, and preliminary values of $\mu = 1.1$ nuclear magneton and $Q = -1.7$ barns. Uranium-235 has $I = 7/2\ (h/2\pi)$(95), $\mu = -0.8$ nuclear magnetons (*94*), and $Q = 8$ barns (*94*). Uranium-233 has $I = 5/2\ (h/2\pi)$ (*55, 94*). Grating spectrograms reveal (*6*) that neptunium-237 has $I = 5/2\ (h/2\pi)$, and neptunium-239 has $I = 1/2\ (h/2\pi)$. A mechanical moment of $I = 1/2\ (h/2\ \pi)$ has been twice reported (*6, 55*) for plutonium-239. It appears that Russian scientists are now beginning to confirm (*55*) the earlier results of others. Grating spectrograms have shown (*20*) that both americium-241 and americium-243 have the same mechanical moments, $I = 5/2\ (h/2\ \pi)$, and that their magnetic moments are very nearly equal.

ISOTOPE SHIFTS

All natural elements, excepting about 20, consist of two to 10 isotopes, and the different isotopes of any given element emit slightly different spectra whose separations are measured as isotope shifts. The compilation and interpretation of such shifts was reported in previous reviews (*64–67*). Their interpretation is still far from satisfactory so their observation continues. The availability of enriched isotopes of high purity has given new impetus to these investigations. Here details of experimental procedure and results will be omitted; only some isotopes for which spectral shifts have been recently measured are listed. Li^{6}, Li^{7} (*43*); *B*^{10}, B^{11} (*14*); O^{16}, O^{18} (*93*); Ca^{40}, Ca^{48} (*76*); Sn^{112}, Sn^{114}, Sn^{116}, Sn^{118}, Sn^{120}, Sn^{122}, Sn^{124} (*41*); Ce^{136}, Ce^{138} (*4*); Nd^{142}, Nd^{144}, Nd^{146}, Nd^{148}, Nd^{150} (*74*); Hf^{178}, Hf^{180} (*17, 26*); Hg^{200}, Hg^{202} (*27*); Pu^{239}, Pu^{240} (*91*).

The precision measurements in the Ge I spectrum deserve special mention because they were made with an atomic beam source (*23*) emitting the sharpest possible lines. The three principal isotopes of germanium with masses 70, 72, and 74 are about equally abundant, which is a favorable condition for measuring small line shifts. No shifts could be detected, and this implies that the volume shift must be small and in the opposite direction to the mass shift.

Another interesting puzzle is found in the isotope shifts between cerium isotopes. Although a shift between cerium-140 and cerium-142 was found and confirmed, even with enriched isotopes no displacement of cerium-136 or cerium-138 lines could be detected (*4*); both lines seem to coincide with cerium-140. These anomalies have not been explained.

SPECTROCHEMICAL ANALYSIS

The literature on applications of atomic emission spectra to chemical analysis has been almost completely compiled up to 1951 (*84*) with a list of more than 3600 publications. Some additional references for the years 1950 to 1953 were given in the last two reviews (*66, 67*). As it is neither possible nor desirable in the present review to itemize the voluminous literature of the past 2 years, only selected items are mentioned here.

Several books on spectrochemical analysis have recently been published, mostly in foreign languages (*3, 60, 63, 71*).

Elaborate electronics are being imposed on light sources to improve them for spectrochemistry. For example, descriptions are now available of an electronic control mechanism for a low-voltage spark generator (*12*), the electronic regulation of the charging potential of condensers in spark generators (*62*), and an electronically controlled spectrographic low-voltage arc, interrupted arc, and direct current arc source (*5*).

Attention is called to a "constant temperature arc" method developed (*2*) at Philips Research Laboratory in Eindhoven; tables of empirically determined ratios of concentration-intensity for 38 elements are given so that they may be checked by other laboratories.

Applications of spectroscopy to steel analysis are now worldwide and almost fully automatic and instantaneous (*39*). The selection of line pairs for the spectrographic analysis of steel has been studied systematically to guarantee high accuracy and reproducibility even under conditions of considerable matrix variation (*18, 30*). Ferrous alloys are continuously becoming more complex; now cerium metal and its alloys and compounds are being used in the production of ductile iron, cast steel, and some stainless steels. The amounts of cerium added are generally of the order of 0.01 to 0.10%. The cerium can be determined spectrographically following either chemical or electrolytic removal of the major interfering components (*80, 86, 89*). Also a direct spectrographic determination of cerium in cast iron has been reported (*47*).

Notable applications of spectrochemistry to biology are the following: a spectrographic study of inorganic elements in human tissues (*15*) shows that copper is a normal constituent and has its greatest storage value in liver. The average values for various metals in milligrams per 100 grams of dry tissue were: liver, copper 2.76, zinc 21.92, iron 47.66, manganese 0.532, and lead 0.517; kidney, copper 1.77, zinc 19.04, iron 27.38, manganese 0.301, and lead 0.362; heart, copper 1.83, zinc 11.85, iron 23.39, manganese 0.147, and lead 0.195; brain, copper 2.07, zinc 4.93, iron 20.47, manganese 0.132, and lead 0.082; lung, copper 1.21, zinc 8.63, iron 103.34, manganese 0.117, and lead 0.234; spleen, copper 1.03, zinc 8.57, iron 112.45, and lead 0.275. Surely every human being is interested in these figures.

The metal content of skin, hair, and nails from white males 15 to 70 years of age was determined spectrographically for 14 metals (*34*); variations are so great that small differences resulting from metabolic disorders cannot be detected by spectrography at this time. If interested, white males between the specified age limits should consult original publication for further details.

The amounts of microelements in the nuclei of nerve cells as measured (*96*) by emission spectra show some striking differences between the ash of the human cerebral and cerebellar cortex—for example, among 17 metals measured, aluminum is 500 times more abundant in the cerebellum than in the cerebrum, while titanium is 10 times more abundant in the latter. The biological role of many of the metals in the animal organism still remains unexplained.

A spectrographic method was employed (*75*) to measure 12 trace elements in the livers of cancer victims and of persons dying of noncancerous diseases; a significant increase (112%) in zinc was found in the uninvolved portion of the liver in all cases with metastatic malignancy.

Spectroscopic determination of gases is relatively rare but usually noteworthy. A new method for determining oxygen in steel was developed (*81*); it uses a discharge tube with a hollow graphite cathode in which the sample is melted by a discharge in argon, liberating the oxygen which combines with graphite to form carbon monoxide. The intensity of the carbon monoxide bands with respect to the argon lines is a measure of the oxygen in the steel.

It has been reported (*59*) that a spectrochemical determination of argon in air can be made in one minute with a precision of ±0.43% at the 95% confidence level.

Six methods of analyzing sea water spectrographically have been tested (*42*); they are identified as rotating disk, flame-excitation, porous-cup, impregnated-electrode, open-cup-residue, and briquet-excitation methods. It is demonstrated that quantitative determinations of the metallic ions, sodium, magnesium, calcium, potassium, strontium, and aluminum may be made by direct examination of sea water or residual salts; the concentrations of these range from 10,000 p.p.m. of sodium to 0.58 p.p.m. of aluminum. Recourse to special separation methods is necessary for spectrographic determination of trace elements iron, copper, barium, lithium, silver, and any others whose concentrations are less than that of aluminum or whose detectabilities are seriously affected by the presence of major sea-water cations.

The popular porous carbon cup technique for spectrochemical analysis of solutions has been facilitated by the publication (*99*) of tables of atomic and molecular spectra always produced by electrical discharges between graphite electrodes in air. Nearly 200 atomic lines belong to successive spectra of carbon, nitrogen, or oxygen and 57 band heads are identified with C_2, CN, NH, or OH.

The flame photometer grows in popularity for the determination of alkalis and alkaline earths; this is shown by review articles with bibliographies (*61, 82*), and recent papers too numerous to mention.

To avoid leaving the impression that spectrochemical analysis is entirely mundane, mention should be made of some recent applications in astrophysics. The relative abundances, by numbers of atoms, of sodium, magnesium, aluminum, calcium, chromium, iron, nickel, copper, and strontium in the sun's chromosphere have been derived (*83*) from recent calculations. The abundance of beryllium in the sun has been derived (*36*) from photometric measurements of Be I and Be II lines. Methods for the quantitative analysis of the atmospheres of stars have been discussed (*33*); from experimental values for the total absorption in all the spectral lines, the quantum number of the last separated Balmer line, and the approximate temperature, it is possible to find, by successive approximation, the number of atoms of each element per square centimeter of the photosphere, the concentration of electrons, the electronic pressure, the ionization temperature, and the effective gravitational acceleration. Further examples can be found among papers presented (*70*) to the fifth international colloquium on astrophysics.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Fluorometric Analysis

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This review covers the 2-year period since the previous review (*91*), from approximately August 1953 to August 1955. In accordance with the policy of this journal, only a selected group of references are included.

APPARATUS

Anyone concerned with selecting a source of ultraviolet light will find an article by Doede and Walker (*22*) very helpful. These authors compare the ultraviolet radiation of about 50 commercial lamps. A rating is given for the lamps in five different parts of the spectrum from 2200 to 3800 A.

A number of research groups during this period have built fluorometers with some special features. Galvanek and Morrison (*39*) report success with the use of the 360 BL type of fluorescent lamp in a fluorometer for the determination of uranium. An electron multiplier phototube and a vacuum-tube voltmeter are used in the detection unit. Kinser (*58*) has described another model of the Geological Survey transmission fluorometer in which he uses a General Electric R. P. 12 lamp for excitation and an end-window photomultiplier tube in the detection unit. Another instrument, designed by Kelley and Hemphill (*57*) for the uranium determination, uses two AH4 lamps at a 45° angle to the sample and a 6199 photomultiplier tube. Florida and Davey (*35*) have also given details for a photomultiplier fluorometer of the reflector type for the measurement of the fluorescence of solids in which the exciting source is at about a 45° angle and the photomultiplier tube is at a 90° angle with the sample. Lynch and Baumgardner (*63*) have designed a fluorescence photometer for use with both solids and liquids, in which both the known and unknown samples are irradiated; the light from each falls on separate phototubes and the ratio of the photocurrents is measured. Dowdall and Stretch (*24*) prefer a 12-volt tungsten lamp over the mercury vapor lamp as an exciting source in a twin-beam null point photomultiplier fluorometer for the analysis of liquids. This lamp is reasonably rich in the longer ultraviolet rays, and many solutions are activated by rays just below the visible region of the spectrum. Niki and Shirari (*70*) describe an instrument for measuring the fluorescent spectra of solids where a photomultiplier tube is attached to a spectroscope.

The photomultiplier tube on the Beckman DU spectrophotometer makes this instrument much more adaptable to the measurement of fluorescence and fluorescence spectra. A very simple modification of this instrument which adapts it for either of these purposes is described by McAnally (*64*). In this case the lamp housing is replaced with one in which the lamp is removed and the solution may be inserted at the focal point of the mirror. Another modification of the Beckman Model DU spectrophotometer places the fluorescent solution at the exit slit and a photomultiplier tube at the light entrance aperture (*42*). Brown and Marsh (*14*) use a modified Beckman Model DU to measure the fluorescence of paper chromatograms. A strip transporting mechanism and a chart recorder are among the attachments used; with a 1P28 tube a concentration of 2.6 γ of riboflavin can be detected. A combination of the Beckman DU and the Spekker fluorometer is described by Swann (*83*). Several new commercial fluorometers which employ photomultiplier tubes have been announced. One of these (*54*) is designed to measure the fluorescence of solids only and uses a fluorescent lamp as the activating source. Another commercial instrument is called a polyfluorimeter (*3*) and is designed to measure the fluorescence of solutions or solids by transmission, reflection, or at right angles. The light source for this fluorometer is an H 100 A 4 lamp, and the photomultiplier tubes 931 A, 1P28, and 1P21 may be easily interchanged. A third commercial fluorometer (*76*), which also provides for a selection of photomultiplier tubes, is designed to accommodate several different volumes of solutions. A combination of two standard monochromators with a photomultiplier tube is offered by another apparatus company (*26*) for the measurement of fluorescent spectra and fluorescence excitation as well as for routine determinations.

Bowman (*11*) and his associates have developed an instrument called a spectrophotofluorometer which is designed to excite and measure the fluorescence in either the ultraviolet or visible part of the spectrum. This instrument employs a Bausch & Lomb monochromator to analyze the excitation from a xenon arc source and a quartz prism spectrograph to scan the fluorescence. The fluorescence intensity is measured with a 1P28 photomultiplier tube coupled to a cathode ray oscilloscope.

A review on fluorometry which includes general principles, instruments, solvents, and the effect of various conditions has been published by Indemans (*52*).

INORGANIC

Fluorescent x-ray analysis is an important phase of inorganic fluorescence analysis, but is not reviewed here because of the attention given this subject elsewhere (*4*, *62*). The increasing application of inorganic fluorescence analysis is indicated by the inclusion of many fluorescence confirmatory tests in collegiate qualitative analysis. In a book by Charlot (*17*), many fluorescence tests are given an equal place with colorimetric reactions. A reference bibliography on fluorescence analysis with 180 titles has been compiled by Konstantinova-Shlezinger (*59*). A very informative article on new fluorescence reagents for aluminum has been published by Holzbecher in Prague (*47*). This author has tested aluminum for fluorescence with compounds of the type of salicylaldehyde, 2-hydroxynaphthylaldehyde, and 18 of their derivatives. Salicylidene *o*-aminophenol at a pH of 5 proved to be one of the best of these. The sensitivity is reported as 0.005 γ of aluminum; the usual fluoride interference is noted.

The fluorescence determination of aluminum and gallium with 8-quinolinol in chloroform has been in practice for some time, and now Collat and Rogers (*19*) have shown that these two elements can be determined in the same chloroform extract. The above authors show that the complexes of these two elements give a different response upon irradiation at two different wave lengths and with the use of predetermined curves or equations they are able to calculate the quantity of each element present in a mixture. This presents a new technique for fluorescence analysis and may have some interesting possibilities. In this case the fluorescence spectra of the two complexes are essentially the same, but the fluorescence responses at two activation points show a difference. In expressing the fluorescence intensities these authors use a quinine equivalence, which is a good technique and will permit others to check the experimental work.

Another possibility for the determination of gallium or indium in the presence of aluminum is presented by Patrovsky (*73*). In this method morin (2',3,4',5,7-pentahydroxy flavone) is used as an indicator for the titration with the disodium salt of ethylenediaminetetraacetic acid; the fluorescence of morin disappears at the equivalence point of gallium and indium. If aluminum is present, fluoride ion is added to extinguish the fluorescence of the aluminum-morin complex. It is of interest to note that morin is advocated as a colorimetric reagent for the determination of aluminum and 1 to 1 ratio of morin to aluminum is indicated (*86*). The authors did not have the equipment for a satisfactory fluorescence determination. Previous reports have indicated that the morin-aluminum composition of the fluorescent complex was 3 to 1.

Toribara and Sherman (*87*) have concluded that morin is the most sensitive reagent for beryllium, which they have tried for the determination of micro quantities of beryllium in organic matter. A new reagent for beryllium, 2-(*o*-hydroxyphenyl) benzothiazole, has been suggested by Holzbecher (*48*). The reagent is 0.001*M* in 96% alcohol, and the test is performed in an acetate buffer at a pH of 4.5. The fluorescence is bluish white, and several metals interfere, such as zinc, aluminum, antimony, and colored ions.

The use of the 8-quinolinol–aluminum complex as a qualitative reagent for fluoride has been tested by Harrigan (*44*) and is recommended over other methods. The test is performed on paper which has been dipped in a chloroform solution of the complex and dried. This coated paper held in hydrofluoric acid fumes becomes nonfluorescent. Bouman (*10*) has successfully used the aluminum-morin chelate for the determination of fluoride in concentrations of 0.02 γ per ml. and advocates that the reaction be performed in 10% ethyl alcohol.

An interesting fluorometric method for the determination of cadmium is reported by Evcim and Reber (*25*). The method involves the precipitation of cadmium with 2-(*o*-hydroxyphenyl) benzoxazole. The precipitate is filtered and washed, the cadmium complex dissolved in glacial acetic acid, and the fluorescence measured. Concentrations of cadmium from 0.1 to 2.5 mg. per 50 ml. were used. This method is of particular interest because the reviewer believes that it illustrates the use of a fluorescent reagent which is restored, in glacial acetic acid, and the fluorescence of the original reagent rather than the complex is measured.

Brodaks, Feigl, and Hecht (*13*) have described a new application for the determination of gallium. Gallium is precipitated with a mixture of morin and cupferron and is extracted from a hydrochloric acid solution with chloroform. As little as 1 γ of gallium in 6 ml. of chloroform is easily determined from a mixture containing 1000 times more aluminum, zinc, and iron. A qualitative fluorescence method for gallium with rhodamine B as the reagent has been developed by Onishi (*72*). This reagent gives an orange red fluorescence that will detect 0.1 γ of gallium, and only a few elements interfere.

Raju and Rao (*80*) have indicated that benzoin will serve as a fluorescence reagent for the detection of germanium. The reaction medium is alkaline 95% ethyl alcohol; the fluorescence is greenish yellow, and 10 γ can be detected in 5 ml. of solution. As these conditions are identical with those used in a very delicate test for boron, the reviewer does not believe that this test for germanium will be very successful. It does, however, suggest the similarity of germanium and boron, and tests for either element should be tried for the other. These same authors (*79*) show that resacetophenone in concentrated sulfuric or phosphoric acid gives a greenish gold fluorescence with germanium, whereas the boron fluorescence is blue. The limit of the test is 100 γ of germanium.

Feigl and Gentil (*31*) have discovered that tin hydroxides form a blue-green fluorescence on paper with morin, which is stable in dilute acetic acid; aluminum, antimony, and zirconium give similar results. The method has been applied to the determination of tin in alloys, minerals, and mordanted silk (*33*).

Fassel and Heidel (*27*) have determined the fluorescent spectra for terbium in terbium chloride solution and have shown that terbium can be determined quantitatively in amounts as low as 0.05 mg. in 10 ml. of solution. Calibration curves are given for terbium in the presence of other rare earths, and the effect of other ions is also shown. This method seems to have distinct advantages over other methods for the determination of terbium and is unique in fluorescence determinations, in that no reagent is added and the intensity is measured at the narrow band fluorescence of the inorganic ion.

The orange-red fluorescence of thallium with rhodamine B will serve to detect 0.03 γ of thallium (*32*).

Pollard and his associates (*78*) have published a series of six articles dealing with the analysis of inorganic ions by paper chromatography, and in this work a number of compounds have been investigated for separating and identifying metallic ions. For example, with the lanthanides it is shown that yttrium, lanthanum, and lutetium give the usual fluorescence with morin and 8-quinolinol but gadolinium fluoresces a deep brown with the latter and green with morin.

Details for the determination of uranium in minerals containing less than 10 p.p.m. are given by Adams and Maeck (*2*). These authors use the sodium fluoride fusion and think it superior to the colorimetric method for small quantities. Zimmerman and Rabbitts (*93*) have reported further on the use of the uranyl fluoride method for the determination of uranium. Walton (*89*) has noted the quenching effect of plutonium and iron in the uranium determination.

A highly selective fluorescence spot test for hydrazine has appeared in the Russian literature (*61*). The reagent is 1 gram of salicylaldehyde in 2 ml. of acetic acid and 100 ml. of water. This mixture is spotted on a drop of hydrazine solution on paper; an orange-yellow fluorescence results and 5×10^{-10} gram of hydrazine can be detected. Ammonia, azides, nitrates, and nitrites do not interfere.

Charles and Freiser (*16*) in the fifth of a series of articles on organic analytical reagents have reported an interesting research on the chelates of 2-(-*o*-hydroxyphenyl)-benzoxazole, 2(*o*-hydroxyphenyl)-benzothiazole, and 2(*o*-hydroxyphenyl)-benzothiazoline. Chelates of the first compound with lead fluoresce green, with zinc blue, and with magnesium blue. The reagent is fluorescent, but chelates with the transition metals do not fluoresce.

A paper of general interest in trace analysis which gives a variety of possible fluorescence applications has been published by Irving and Rossotti (*53*). These authors give a theoretical discussion of sensitivity test and tabulate the results of their experiments on the color and fluorescence of 33 cations with 8-quinolinol, 8-hydroxyquinazoline, and 5,8-dihydroxy-2,3-dimethyl quinoxaline. At present none of these reagents seem superior to 8-quinolinol, but further tests are in order.

ORGANIC

The increasing popularity of fluorescence in organic analysis is indicated by the inclusion of a number of fluorescence reactions in two new books on methods of analysis of organic compounds. Feigl (*28*) has 29 applications of fluorescence and Pesez and Poirier (*75*) have 34. Both of these books are excellent references. Feigl and his associates (*29*) have discovered a sensitive and specific test that will detect 0.005 γ of coumarin. When coumarin is dissolved in alkali, the pyrone ring opens with the production of the alkali salt of *o*-hydroxycinnamic acid which becomes highly fluorescent after exposure to ultraviolet light because of the formation of the trans configuration. Coumarin is removed from impurities by vaporizing it at 100° C. onto test paper moistened with alkali. A rather extensive paper dealing with the separation of coumarin and its derivatives has been published by Berlingozzi and Fabbrini (*9*). Rhodamine B is recommended by Feigl and Gentil (*30*) as a fluorescence reagent to detect enolizable polynitro compounds. These compounds give a red-violet salt with rhodamine B, which gives an orange-red fluorescence in benzene. Stubner (*82*) has shown that DDT can be detected under the microscope by its orange-yellow or lemon-yellow fluorescence.

A 58-page discussion on organic fluorescent and photochemical substances by Fujimori (*38*) will be of general interest to anyone dealing with fluorescence. The article is in English, and the theory of fluorescence as related to absorption and photosensitivity is discussed in some detail. Many absorption and fluorescence spectra curves are included and a great number of specific cases are given. For example, in one section the reaction of anthrone with formaldehyde, glycerol, etc., to produce six fluorescent compounds is illustrated by formulas and curves, and the effect of solvents is shown. Another section of the paper is devoted to the "phototrophy of new photosensitive complexes between a fluorescent pigment and SH compounds."

The previous work of other authors on the separation of hydrocarbon classes in light petroleum distillates by adsorption on silica gel and identification by fluorescent indicators has been extended by Harvey and Pearson (*45*).

BIOLOGICAL

Fluorometric methods for the determination of steroids have been the subject of a number of excellent papers. Bates (*5*), in a paper delivered before the Laurentian Hormone Conference, discussed spectrophotometric and fluorometric methods for the determination of estrogenic steroids. Goldzieher and Bodenchuk (*41*) studied the fluorescent reaction for 29 steroids. These authors list the wave length of peak intensity and the fluorescence intensity at this and several other wave lengths. The influence of isomerism, unsaturation, and hydroxy groups is also included. Sweat (*84*) has made an extensive study of the fluorescence of corticosteroids in the presence of sulfuric acid and has also shown the use of a silica microcolumn for chromatographic resolution of corital steroids (*85*).

Abelson and Bondy (*1*) have described a simple fluorometric procedure for the analysis of Δ^4-3-ketosteroids. This determination is based on the reaction of the steroid with potassium *tert*-butoxide in *tert*-butyl alcohol and is applicable to testosterone, progesterone, and the biologically active adrenocorticol C_2 steroids. Braunsberg (*12*) and his associates have made an extensive study of the fluorescence of estrogens in phosphoric acid and have found it possible to measure, for example, 0.01 γ of estradiol by the use of a photomultiplier fluorometer. Heusghem (*46*) has shown that hydrogen peroxide destroys the fluorescence of estrogens and that the residual fluorescence after the hydrogen peroxide treatment should be subtracted from the original value.

The use of fluorescence in the ultraviolet region of the spectra for the identification and determination of biological compounds has been developed by Udenfriend and coworkers (*11, 88*). These authors have developed instrumentation for activating and measuring fluorescence in the 250- to 650-mμ region of the spectra. Serotonin, for example, is activated at 295 mμ, and its maximum fluorescence is measured at 330 mμ. The authors give the activation wave length and fluorescent maximum for 20 compounds. Experiments indicate that the measurement of fluorescence in the ultraviolet will be an important technique in analytical chemistry.

Serotonin and other tryptamines have been shown by Jepson and Stevens (*55*) to produce an intense blue-green fluorescence on paper when treated with 0.2% ninhydrin in acetone containing 10% by volume of glacial acetic acid. This test is sensitive to 10^{-4} micromole per square centimeter. The spectral fluorescence and absorption of 3,4-benzopyrene has been the subject of a detailed study by Berg and Norden (*8*); the fluorescence spectrum changes from violet to white-yellow with concentration and solvents. The fluorescence of pyridine nucleotides is affected by calcium and magnesium ions, and the depression of the fluorescence by these ions in the buffer becomes an important factor in the analysis of nucleotides (*60*).

The estimation of alkaline phosphates on blood serum has been simplified by the use of fluorescence for the analysis of 2-naphthol (*56*). Freytag (*36*) has developed a new fluorometric method for the determination of ascorbic acid and mercaptan compounds which is based on the reduction of 1,2-naphthaquinone-4-sulfonate. An article in Japanese, but with an English summary, also deals with the determination of vitamin C (*71*).

Interesting factors affecting the fluorometric determination of *N*-methyl nicotinamide have been found by Rosenthal (*81*). Pretreatment of the samples with alkaline or neutral peroxide may completely destroy the fluorescence and many elements catalyze the formation of the fluorescent derivatives; iridium and cerium salts are especially effective. A fluorometric determination of cholic acid which is said to be specific has been described by Pesez (*74*). The procedure for the determination of gitoxin has been improved by the addition of ethyl alcohol to the extraction media (*37*).

A simple method of separation of vitamins B_1 and B_2 by paper chromatography and the use of fluorescence to mark the cut and estimate the material in each band is described by Giri and Balakrishnan (*40*). The fluorescent bands are cut from the paper and eluted for the final measurement of the fluorescence. A modified fluorometric procedure which is sensitive to 5 γ per liter of adrenaline and noradrenaline is contained on a report from the Mayo clinic on the quantitative determination of these materials (*65*).

The fluorescence and color intensity of antimony trichloride with bile acids may be used for the identification of a number of cholanic acids (*15*). The fluorescence is specific for the position and number of hydroxyl or keto groups attached. Neu and Hagedorn (*69*) have shown that antimony trichloride is a better reagent than boric acid for the determination of the flavonols. These authors have tabulated the color and fluorescence of morin, quercetin, rutin, and others with this reagent. Horhammer and his associates have published a series of articles (*43, 49–51*) dealing with the separation, identification, and characteristics of the hydroxyflavones. The authors have studied in detail the reactions of boric acid and zirconyl ions with these compounds. Tables are given for the color and fluorescence of the zirconium and boron hydroxyflavone complexes, and evidence is presented to show that the hydrogen of the hydroxyl in the number 3 position is replaced by zirconium. Neu (*68*) has developed the procedure necessary for the use of boron as tetraphenyl diboronoxide as a color and fluorescence reagent for the hydroxyflavones and 8-quinolinol.

Miller and Johnson (*67*) have presented a new fluorometric method for the determination of tryptophan, in which glucose reacts with tryptophan and the fluorescence of the resulting substance is measured. The detection of adrenaline and noradrenaline on paper chromatograms is simplified by a reaction reported by Pitkanen (*77*). Potassium ferricyanide is used as an oxidant and the chromatogram is then treated with dimethylaminobenzaldehyde; adrenaline becomes dark blue and noradrenaline is changed to a yellow compound with a strong yellow fluorescence which fades as a blue color forms.

The absorption spectra and fluorescence spectra of certain pigments of the porphyrin group have been published by Crow and Walker (*21*) in connection with a study of laboratory products isolated and purified by chromatographic and electrophoretic analysis. Becker and Kasha (*6*) give data on the fluorescent band of etioporphyrin II and its zinc complex, phthalocyanine and its magnesium complex, and pheophobide-a and chlorophyll a and b. Low temperatures narrow the fluorescent bands and give peak values which may be of use to the analyst. The separation of porphyrins by paper chromatography and their detection by fluorescence have been described by Corwin and Orten (*20*).

An Amberlite column previously treated with sodium hydroxide has been used to separate fluorescent impurities from vitamin B_1; the B_1 is then eluted with hydrochloric acid (*23*). As water solutions often elute fluorescent materials from resins, a careful check on blanks must be made when this procedure is used.

A fluorescent method is favored by Bencze (*7*) for the determination of small amounts of vitamin E. In this method the tocopherol red is condensed with *o*-phenylenediamine in acetic acid, and the resulting phenazine derivative is adsorbed from a light petroleum solution on to activated alumina. The compound is eluted from the column with a benzene-petroleum mixture and is taken up in a methanol-butanol mixture and its fluorescence is measured. Cahmann (*18*) has published excellent curves for the fluorescence spectrum of benzo-[α]-pyrene and has shown the importance of both fluorescence and absorption measurements in the determination of this compound in shale oil.

Lawrence and his associates have reported on some interesting cases of substances which are not fluorescent in water but become highly fluorescent when adsorbed on paper (*34*) and have applied this principle to the determination of proteins (*90*). For example, 1-*anilinonaphthalene-8-sulfonic* acid is nonfluorescent in aqueous solution but becomes brightly fluorescent when adsorbed on protein molecules. The fluorescence of blood plasma containing an excess of this dye was shown to be a linear function of the albumin concentration. In the naphthalene and acridene series the introduction of an aniline group results in a compound nonfluorescent in aqueous solution but brightly fluorescent when adsorbed on a solid.

Mavrodineanu (*66*) and his associates have shown that fluorescent materials on paper chromatograms separated from plant materials can be measured with a simple adaptation of a standard transmission density unit of the Photovolt Corp. photomultiplier photometer. The lower limit of measurement for indole-3-acetic acid was about 0.5 γ per spot. A review of the use of the photoelectric fluorometer and its biological applications is given by Yagi and Tabata (*92*) with 24 references.

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Polarographic Theory, Instrumentation, and Methodology

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LINGANE (*170*) in the last previous review of this topic commented on the rapid growth of the polarographic literature. During the ensuing 5 years, the number of publications has shown no indication of leveling off; in fact, the growth appears to be exponential. Well over 2000 papers have appeared in this period and the present rate of growth of the literature appears to be in excess of 600 titles per year. This rapid expansion makes all the more imperative a selective type of review, and the policy of considering a limited number of subjects and publications, set in prior reviews (*169, 170*), is being continued. The choice of topics is somewhat arbitrary and inevitably tends to reflect the reviewer's interests.

An outstanding event in the literature of the subject has been the appearance of the second edition of Kolthoff and Lingane's "Polarography" (*140*), now expanded to two volumes. This work, the "Bible" for English-speaking polarographers, will continue to be the standard work of reference for many years to come. Müller's monograph, long a favorite with beginners in the field, is also available in a new edition (*205*). Intermediate in size and extensiveness of treatment is Meites' "Polarographic Techniques," which contains among other valuable features a chapter on trouble-shooting and a comprehensive listing of half-wave potentials (*192*). For a thorough theoretical treatment of certain aspects of polarography and many related electrochemical methods, one should consult the monograph by Delahay (*43*).

Of tremendous aid in searching the literature are the comprehensive bibliographies prepared by several workers. Heyrovský and his associates have published a complete bibliography for the years 1922 to 1950 (*104*), and this is kept up to date by annual supplements in the *Collection of Czechoslovak Chemical Communications* (*103*). The very good bibliography of Semerano (*241*) has likewise been kept up to date by a series of collaborators (*68, 198–200*).

It is regrettable that the two bibliographies of polarographic literature issued by American manufacturers of polarographic equipment have not been kept up to date. One of these (*164*) has not been revised during the past 5 years and the other (*236*) is 10 years out of date. This is in embarrassing contrast with the enterprise of the European manufacturers, who support quarterly journals devoted to polarography. *Radiometer* in Copenhagen publishes abstracts and short articles (in English) under the editorship of P. Kivalo (*130*) and Leybold is responsible for *Polarographische Berichte*, which publishes original articles and critical abstracts under the editorship of Hans and Bergmeyer (*88*). Of the various review articles which have appeared, those of Laitinen (*159*) and Tanford and Wawzonek (*262*) are of particular interest. Wawzonek (*274, 275*) has discussed organic polarography and Laitinen (*160*) amperometric titrations in other reviews published in ANALYTICAL CHEMISTRY. Zuman's list of half-wave potentials is also worthy of particular mention (*283*).

The present review extends from the time of the last previous review at the end of 1950 until late in 1955.

CLASSICAL POLAROGRAPHY

APPARATUS

Instruments. The instruments described in previous reviews continue to be very popular. A new automatic polarograph with pen recording is marketed by E. Leybold in West Germany, and the Fisher Scientific Co. in Pittsburgh now provides an adapter kit for converting the manual Elecdropode into an automatic recording polarograph. The kit provides a drive for the polarizer, and the instrument can be connected either to Fisher's own or to any other suitable potentiometer recorder. A direct-reading, manual polarograph available from the Patwin Instrument Co. is not unusual in its basic design but is made for somewhat more accurate measurements than average. The Fisher Scientific Co. has made available an amperometric titration apparatus to be used with its Elecdropode, and E. H. Sargent and Co. is marketing an electrometric titration instrument, called the Ampot for amperometric, polarized electrode, and potentiometric titrations. It may also be used for measuring current-voltage curves.

National Enterprise in Prague is marketing a portable oscilloscopic polarograph, the characteristics of which have been described by Heyrovský (*97*, *98*, *100*).

Various workers have described homemade instruments, many of which are conventional in design. One which definitely is not, is the polarograph of Mundy and Allen (*206*), which accomplishes automatic recording without recourse to electric or other motors! Mercury under the force of gravity flows through a constriction in a U-tube, and the gradual submergence and exposure of resistance wires by the changing mercury levels are used to apply the variable potential across the cell. Current recording is accomplished photographically, and the camera drive is actuated by the fall of a plummet floating on the descending mercury column as the potential is applied. The polarogram is recorded on a circular disk of sensitized paper, which rotates about an axis through its center. Potential corresponds to angular displacement from the starting point and current to linear displacement from the central axis. This particular apparatus does not appear to recommend itself for general use, but the authors are to be commended for having displayed truly remarkable originality and ingenuity.

An unusual manual polarograph has been described, in which diffusion currents are measured potentiometrically using a cathode ray tube for detecting balance. This instrument is also capable of giving derivative polarograms and may be used to measure pH with a glass electrode (*111*). Wåhlin (*272*) has described a polarograph which records during only a selected part of the life of each drop. In view of the current *vs.* time studies mentioned below, this arrangement may possess very real advantages.

Kelley and Miller (*125*) have described an ingenious, high-sensitivity recording polarograph for use with extremely low concentrations. A 1-mv. Brown recorder is used with a preamplifier so that current measuring resistances up to 1 megohm can be utilized without undue sluggishness of response. A linear compensator is provided for bucking out condenser current, but the most novel feature is a curve-follower to eliminate the effects of residual current. A preliminary polarogram is run on the supporting electrolyte and this trace is transferred to the curve follower, which reads and automatically subtracts the residual current during the running of the actual polarogram. The authors show results on lead solutions as dilute as $2 \times 10^{-7}M$, the diffusion current being of the order of 1×10^{-3} μa. They point out that precision is poor at these levels (about 20%) and that polarography is being pushed to its limits. The reviewer might add that polarography at these levels, even with this type of apparatus, is very difficult to do. After having this type of apparatus in his laboratory over a period of time, the general consensus among those using it was that although good results could be obtained under favorable circumstances, other lines of approach were more promising for the analysis of submicromolar solutions.

Accessories. Among the many minor modifications which may be made to conventional polarographic apparatus to provide added accuracy or convenience, the following should be mentioned. Streuli and Cooke (*259*) suggest the substitution of a multiturn Helipot in place of the polarizer of the Leeds & Northrup Type E Electrochemograph, in order to smooth out small voltage fluctuations caused by lack of enough turns on the winding of the polarizer. This device has the additional advantage of allowing free choice in the rate of polarization, ordinarily fixed on this instrument. Hume and Gilbert (*108*) have suggested a simple "wave spreader" which permits the setting of any desired initial potential with the Sargent Model XII polarograph. With the aid of this device it is possible to spread out the wave for close examination or a careful measurement of half-wave potential, regardless of its position on the potential scale.

Gordon (*83*) has described a peak-current recorder from pen-recording polarographs. This is simply an extra pen riding above the galvanometer pen but not following it back from its maximum excursions. Dependence upon this device as a means of smoothing curves for routine use may lead to serious trouble if maxima or minima appear on the polarograms. The use of a cathode ray oscilloscope as a null point detector in a Wheatstone bridge in measuring the resistance of polarographic cells has been suggested (*219*). Much the same advantages are obtained if one uses an alternating current conductivity bridge with a cathode ray tube balance indicator, the kind commonly and commercially available. By watching the opening of the cathode ray tube, it is possible to measure the resistance of the cell at the maximum drop size, a factor important in precision measurement of half-wave potentials.

Nicholson (*208*) has described a simple arrangement of a Helipot coupled to the potentiometer-recorder to feed back a voltage compensating for the internal resistance drop in polarograms. Meites and Sturtevant (*197*) have designed an automatic drop timer for use with the dropping mercury electrode. The life of an individual drop may be measured with high precision, or the average life of many drops may be determined. Antweiler (*5*) has made an ingenious suggestion to overcome a principal drawback to photographic recording of polarographs—namely, the necessity of development in a dark room. He uses ultraviolet-sensitive paper which is not seriously affected by room light and replaces his galvanometer illumination with a high pressure mercury lamp.

Electrodes. A number of new variations of familiar dropping electrode designs have appeared: a dropping electrode with a constant head (*158*), a dropping electrode in which the mercury comes in contact only with glass (*105*), and a modification in which a relatively small amount of pure mercury is used and ordinary mercury, carefully kept from contact with the pure mercury, supplies the pressure (*110*). Von Stackelberg has suggested silicone treatment for capillaries to improve their characteristics (*253*) and Müller has provided a useful nomogram showing the relation of pressure and drop time to length and radius. By means of this nomogram it is possible to select capillaries of known drop time if the radius is known (*203*).

Smoler (*247*) has described a bent dropping electrode which presents a vertical face. This type of electrode is characterized by a very short drop time, reducing galvanometer oscillations. The characteristics of such a capillary appear to be satisfactory and the author shows data for the linearity of the diffusion current with concentration and with the square root of the head of mercury. Wåhlin (*272*) has described another electromagnetic hammer device for dislodging drops, and Stankoviansky (*255*) has devised a motor-driven chopper for a battery of six dropping electrodes. The "revolver electrode," as it is called, is similar to previously described multiple dropping electrodes, except that the revolving chopper provides equal drop time for each electrode.

Oscillations of the galvanometer are virtually eliminated and the diffusion current is increased by a factor of 6 over that of a single electrode. The increase in actual sensitivity, however, is not as great, because the residual current is increased correspondingly.

Cells. Probably no aspect of polarography has evoked more individuality than the design of polarographic cells. Every year produces a large collection of original, and not so original, variations. The H-cell is one of the most popular forms and several modifications have been described: a micro H-cell (*194*), a cell with two sintered-glass plugs in the cross arm and provision for replacing the intermediate solution to prevent contamination of the test solution with that in the reference electrode (*218*), a cell with a lengthy connecting passage likewise designed to avoid contamination of the test solution (*193*), and a thermostated H-cell in which not only the test solution but also the reference electrode is water-jacketed (*143*). Some authors prefer elaborate assemblies, which are frequently all-glass (*25, 60, 64, 246*); these are in striking contrast with the preference of many polarographers for something which consists of only a beaker and a multiholed rubber stopper. Among the many suggestions in the literature, one which in the reviewer's experience has considerable merit is that of Gawron (*75*), who uses a viscose or cellophane membrane to separate the test solution from the salt bridge. The author has found this to be a very simple and inexpensive expedient for preventing contamination with ions from the reference electrode, and even two of these membranes used in series do not contribute seriously to the total cell resistance. A modification of Gawron's cell has been described by Ševák (*243*). Another technique which has found ready acceptance in the reviewer's laboratory is Carson's (*28*) device of using porous glass (leached but unfired Vycor), impregnated with electrolytes, as a salt bridge. Bridges made in this way have low electrical resistance and provide negligible fluid flow. It is sometimes necessary to leach residual iron from the porous glass rod before use but the author has encountered no other difficulties. The authors also recommend the use of ion exchange membranes as salt bridges.

Ladisch and Knesbach (*157*) have designed high surface-area calomel electrodes by confining mercury in a glass frit. These electrodes have better reproducibility than the ordinary saturated calomel, which may change its potential by about 1 mv. if the platinum contact becomes wetted with the solution. Brunner and Means (*24*) have described a simple and compact plug-in type of calomel electrode similar to those used with commercial pH meters, but having the much lower resistance necessary for polarographic use. Readers consulting their paper should note that the illustration given with it does not apply; the proper one appears as a correction in a subsequent issue of the same journal.

THEORY

General. A number of authors have been considering the interrelationships among various electrochemical methods from a rather broad standpoint. Polarography is but one of many methods, and it is instructive to examine its place in the field as a whole. Reilley, Cooke, and Furman (*225*) have described an ingenious three-dimensional model for interpreting electrometric processes. This surface, in which current, potential, and per cent oxidized are the coordinates, provides a unified basis for relating polarography, voltammetry in general, potentiometry, and polarized end-point phenomena that has been useful in correlation and prediction of new applications. Kolthoff has clarified many of the relations between voltammetry and potentiometry (*134*) and Gauguin (*72*) has discussed the use of polarization curves to describe and predict the behavior of electrodes in various processes. Vlček (*270*) has begun an interesting series of papers on the relationship between the electronic structure of inorganic depolarizers and their polarographic behavior.

Half-Wave Potential. Although it had been recognized for a long time that half-wave potentials are a function of ionic strength by virtue of the fact that activity coefficient of the reducible or oxidizable ion appears in the equation of the wave, it remained for DeFord and Andersen (*38*) to demonstrate how large the effect might actually be. In a study of the half-wave potential of cadmium in nitrate supporting electrolytes where complex formation is extremely weak, if it exists at all, they observed that all nitrates tended to give the same value of cadmium half-wave potential in very dilute solution, extrapolation to infinite dilution giving a standard half-wave potential analogous to the standard potentials in potentiometry. At ionic strengths above a few tenths molar, the half-wave potential became dependent upon the cation and above $1M$ it is important that not only the ionic strength but also the particular supporting electrolyte be specified in the precision measurement of half-wave potentials. The plot of half-wave potential as a function of the square root of ionic strength has a striking resemblance to the plot of activity coefficients as a function of ionic strength, as would be expected from the equation of the wave.

Hershenson, Smith, and Hume (*96*) confirmed these observations in a study on the effect of ionic strength and supporting electrolyte on the half-wave potential of lead in perchlorate media and showed, by use of cells without liquid junctions, that the differences could not be attributable to liquid junction potentials. It must be recognized in precise polarography that differences of 5 to 35 mv. in half-wave potentials are easily possible, owing to ionic strength and specific ion interactions, even in the absence of complex formation.

Vlček (*269*) has recently begun an extensive project on the precise measurement of half-wave potentials and their extrapolation to infinite dilution. He has discussed and evaluated some of the previously suggested methods for the precise determination of half-wave potentials, and pointed out the great advantage to using a third half-cell through which no current flows as the reference for measuring cathode potential. This method has also been discussed by Gardner (*69*), who pointed out that the idea is by no means new. Vlček (*268*) recommends a modification of the interpolation method of Meites (*185*), although he feels that Meites' method is some tenfold less accurate than was originally claimed. Weber (*278*) has suggested a least-squares method of estimating half-wave potentials. This approach is likely to give an entirely erroneous notion of the precision and accuracy of the measurements for, in common with most of the estimates hitherto made on these quantities, attention is paid only to the fit of the data on a particular wave. Far more significant is the run-to-run variation where replicate solutions are electrolyzed with complete resetting of all instrumental adjustments. The reviewer has always preferred to estimate half-wave potentials, where a precision 1 mv. or better is desired, by interpolation from a plot of log $[(i_d - i)/i]$, correction for residual current and iR drop having been made (*107*). When a pen-recording instrument is used, the recorder should be undamped, the maximum pen excursions used as the measurement of current, the wave well spread out, and the potentials at the beginning and end of the polarogram carefully measured with an accurate potentiometer. Half-wave potentials may be estimated to a precision of about ±0.1 mv. in this way and the run-to-run variability is the order of 0.5 to 1.0 mv. In view of the larger run-to-run variability, it is often sufficient simply to estimate the half-wave potential from a well-spread-out curve (*63*). Markus (*179*) has described a method of correcting for iR drop without measurement of resistance by measuring half-wave potential as a function of concentration, assuming the inconstancy in half-wave potential to be due only to iR drop, and extrapolating $E_{1/2}$ to $iR = 0$.

Vlček (*271*) has described a method of comparing half-wave potentials in different solvents and suggests the use of potassium as a reference element, assuming its solvation energy to be negligible in most media. Kamecki and Suski (*124*) have made studies on the relationship between half-wave potential and temperature. As their results are not in agreement with present theories, it will be of interest to see which is vindicated by further investigation. Kivalo (*131*) has re-examined the principle of Müller's oscillographic method for measurement of half-wave potentials and made a refinement in the theoretical interpretation.

Diffusion Current. At the time of the last review, a great deal of interest centered around modifications of the Il'kovič equation. Both Lingane and Loveridge, and Strehlow and von Stackelberg had proposed modifications taking into account the curvature of the electrode surface. Both equations were essentially of the form

$$i_d = 607\, nD^{1/2}\, Cm^{2/3}\, t^{1/6} \left[1 + \frac{At^{1/6}D^{1/2}}{m^{1/3}}\right]$$

but the value of the constant, A, was in the first instance 39 and in the latter 17.

Since that time, there has been an abundance of experimental work devoted to testing the two relationships (*34, 92, 186–188, 190, 196, 204, 231, 252, 258*). The results of these studies tended to support sometimes one, sometimes the other point of view, and sometimes neither. What became abundantly plain was that there are many experimental factors which are important in measuring the very small differences involved. Electrical and mechanical characteristics of the apparatus, such as galvanometer period, may introduce effects of the same order of magnitude as the discrepancies being measured, and migration current must be very carefully eliminated. Maximum suppressors may have a very serious effect on the diffusion current; the general and frequently undesirable effects of maximum suppressors have been discussed by a number of authors (*31, 195, 263*).

A good deal of new insight into the problem has resulted from current-time studies on individual drops (*90, 91, 171*). One of the major causes of divergence from both the original and modified Il'kovič equations is now known to be variation in the rate of flow of mercury during the life of the drop. Back pressure due to interfacial tension is much greater when the drop is small, so that the current is proportionally smaller at the start of the drop than if the flow were constant. For low mercury heads one finds a measurable pause at zero current after the fall of the drop. Another factor which may need consideration is the lowered concentration of reducible material in the solution left behind when a drop falls. Masuda (*181*) takes into consideration the effect of the capillary in shielding the drop, which appears to be important in the early stages of drop growth.

The most recent theoretical studies (*123, 154*) have led to modified Il'kovič equations of the same form as given above, but with numerical values of constant A equal to 30 and 34, respectively, intermediate between the two earlier values. The deviation from the original Il'kovič equation is not large and therefore the differences in the equations are actually small. The phenomena involved are probably too complicated to be exactly expressible, either theoretically or experimentally, by any single, simple equation and it is remarkable that the modified Il'kovič equations hold as well as they do. But however well theoretical or empirical equations may fit diffusion current data, it seems likely that, in view of the simplicity with which standardization curves can be made, the majority of polarographers will continue to use this safe and sure method.

Miller and Orlemann (*202*) have pointed out that, in certain mixtures of reducible species, polarographic diffusion currents are not strictly additive. This results when there are fast reactions between reduced and oxidized species and when the oxidized forms have significantly different diffusion coefficients. In a mixture of ferric iron and dichromate, for example, ferrous ions diffusing out from the electrode reduce sexivalent chromium to the trivalent state. The ferric ions produced have about half the diffusion coefficient of the dichromate ions they replace, and a lowered diffusion current results. This appears to be a general phenomenon of varying importance and should be kept in mind when mixtures are being determined. Gokhshteyn (*82*) has made a study of diffusion of electrolytes by a diffraction method and claims that the diffusion coefficients used in the Il'kovič equation are substantially higher than they should be. If these results are substantiated, it will be necessary to take account of them in the theory. Furness (*67*) has studied the change in potential of the dropping mercury electrode during formation of individual drops. He points out that the potential of the drop is a function of current, and inasmuch as the current varies with the drop life, the potential varies also. As a consequence it is most reliable to measure both current and potential at the maximum drop size.

Limiting Currents Controlled by Reaction Rates. Brdička and Wiesner in 1943 were the first to consider the effect of slow chemical reactions at the surface of the dropping electrode on polarographic limiting currents. During the past few years, there have been very intensive theoretical studies on this and similar problems related to irreversible waves. Investigators in widely separated laboratories, notably Koutecký and Brdička in Prague, Delahay in the United States, and Kambara and Tachi in Japan, have derived very similar theories more or less at the same time. Brdička and Koutecký (*19*) have summarized the historical development of the work.

In any general approach to irreversible and kinetically controlled waves, it is necessary to take into consideration mass transfer both by diffusion and by migration, chemical reaction by rapid and slow reactions of various orders, and the rate of the electron transfer reaction itself. Additional complicating factors in any rigorous treatment include stirring and convection effects, adsorption of both reactants and products, and effects connected with the electrical double layer and the solvation of the reactants and products (*220*). A large number of papers have appeared in which these factors are considered individually and collectively, the theory of kinetically controlled currents being developed particularly by Koutecký (*146, 147, 149–153, 155*). Smutek (*248–250*) has given attention to slow electrode reactions, as have Kambara and Tachi (*122*), who arrived at much the same results.

Studies involving slow solution reactions, slow electrode reactions, or both, have been made by Delahay and his coworkers (*15, 40, 48, 51, 257*), Hush, Oldham, and their associates (*61, 129, 132, 211, 212*), and others (*10, 70, 89, 126, 127, 201, 221*). Most of these papers are mathematical, and for a straightforward and very readable discussion of currents controlled by reaction rates, the reader is referred to the review by Wiesner (*279*), a pioneer in the field, whose early simple theory of kinetic waves has been shown to be essentially correct by Koutecký's more recent rigorous derivations. Jordan (*112*) likewise has a good discussion of reversibility and irreversibility in terms of the competing processes of mass transfer and electron transfer. Brdička (*18*) and Delahay (*52*) have discussed the application of polarography to the evaluation of certain rate constants. Zimmermann and Gropp (*282*) have pointed out the influence of adsorption on the shape of polarographic waves.

The effect of disproportionation of the reaction product at the surface of the electrode has been studied by Orlemann and Kern (*213*) and further examined by Koutecký and Koryta (*145, 156*). The theory of catalytic waves has not been neglected, as may be seen by reference to the papers of Delahay (*41, 50*) and Koutecký (*148*).

Amalgam Electrodes. The behavior of dropping amalgam electrodes has been studied by a number of workers. Because many dilute amalgams are easily oxidizable by air, and because of the tendency of amalgams to adhere to glass, the experimental technique is considerably more difficult than that of ordinary polarography. Furman and Cooper (*66*), however, have studied a number of dropping amalgam electrodes and found it possible to measure the diffusion coefficients of the metals in mercury. When anodic dissolution waves of amalgams are used for analytical purposes, the concentration of a single metal in an amalgam may be determined readily, but mixtures do not give good results. It is suggested that compound formation takes place between different metals dissolved in mercury. Kössler (*133*) has also studied limiting currents due to dissolution of amalgams and derived equations for the limiting current. Tockstein (*264*) has utilized dropping amalgams to study slow, irreversible electrode processes and von Stackelberg and Toome (*254*) have determined the diffusion coefficients of a number of metals in mercury. An attempt has been made (*80*) to use a dropping gallium electrode, but the properties of gallium (rapid oxidation in air, tendency to wet glass, and low hydrogen overvoltage) make it unsuitable for polarography.

METHODOLOGY

MILLICOULOMETRY

The idea of direct coulometric measurement of the number of electrons involved in polarographic electrode processes has occurred to a number of workers. Essentially the technique consists of measuring the total current (directly or indirectly) during electrolysis of a small amount of solution with a dropping mercury electrode. Both the names "millicoulometry" and "microcoulometry" have been applied, although in view of the actual amount of electricity involved, the term millicoulometry would appear to be preferable. The most simple approach is that of Gilbert and Rideal (*81*), who electrolyzed a small volume of an azo dye solution at constant cathode potential for about 45 minutes. The current was recorded continuously and coulombs were estimated from the area under the current-time curve. At the end of the electrolysis the solution was stirred to remove differences in concentration caused by depletion of the solution near the microelectrode and the change in concentration was measured by determining the final diffusion current. The same technique was used by Reynolds and Shalgosky (*230*), who studied the method as applied to a variety of systems.

Bogan, Meites, Peters, and Sturtevant (*17*) described an ingenious electronic coulometer incorporating an integrating relay, and showed its applicability for the determination of n-values for several metal ions. DeVries and Kroon used two dropping electrodes in series, one in a solution to be examined and the other in a solution containing an ion of known characteristics. From the relative changes in concentration the number of electrons in the unknown reaction could be determined very readily (*55*).

Millicoulometry gives the appearance of being a simple and straightforward tool for the determination of n-values, but it has been the experience of the reviewer, among others, that there are hidden variables and unknown factors which must be brought under control and taken into account before the method can be considered to give trustworthy results. Peattie and Hume (*217*) placed an iodine microcoulometer in series with the dropping electrode, and measured the liberated iodine spectrophotometrically in the ultraviolet. In extensive experiments with cadmium in potassium chloride over a range of potassium chloride concentrations, they were able to duplicate Meites' results for an n-value of 2 for cadmium, but on changing the concentration of supporting electrolyte found that values for n could be obtained anywhere from 1.5 to 3.0. In agreement with Reynolds and Shalgosky, they were unable to get results on homogeneous reversible systems. More recently, Collat and Lingane (*32*) have disagreed sharply with Meites' (*189*) millicoulometric conclusion that nitrate was not itself reduced at the dropping electrode in the presence of high-valent cations, and Grabowski (*85*) also by millicoulometric techniques has found hydroxylamine and ammonia as the products of polarographic reduction of nitrate. It is necessary when using this technique to take especial pains to avoid access of oxygen and to separate the falling drops and keep them from entering the working anode.

Scheffel (*239*), who has investigated polarographic reduction products by ultraviolet spectrophotometry, has noted that in many organic systems the reduction product is adsorbed on the falling drops and, if they are allowed to reach the anode, is promptly reoxidized. Meites (*191*) has studied the so-called "water wave" of Orlemann and Kolthoff (*214*) and concluded that it was due neither to reduction of water nor to stirring effects. This conclusion, however, must be regarded as tentative until the reliability of millicoulometry is more firmly established.

DERIVATIVE AND ALTERNATING CURRENT POLAROGRAPHY

Derivative polarography, so called because the rate of change of current with respect to potential is recorded rather than the current itself, has continued to excite interest because of its great advantage in the detection and determination of trace constituents in the presence of much larger concentrations of other substances.

Říha (*232*) has described a simple circuit for automatic recording of derivative polarographic currents using the charging current of a large condenser. He applied it to such problems as the detection of traces of lithium in sodium and pointed out that, although the peak derivative current is only of the order of 5% of the conventionally measured diffusion current, it is possible to increase the sensitivity of the detector to compensate for this. Lingane and Williams (*172*) made a critical and thorough study of the characteristics of the Leveque-Roth circuit and developed a limiting equation for the magnitude of the derivative current maximum for reversible electrode reactions, assuming that charging and recording lag are negligible. In actual practice, observed derivative maxima are smaller than the ideal value, but the theoretical limiting value is closely approached when the time constant of the condenser-galvanometer circuit, the galvanometer period, and the rate of change of the applied e.m.f. are made very small. The derivative maximum is directly proportional to the concentration of the depolarizer only when the concentration and the resistance in the cell circuit are both very small; otherwise the ratio of the derivative maximum to concentration decreases with increasing concentration to an extent depending on the cell circuit resistance. Charging lag, together with any galvanometer lag, results in an asymmetrical derivative curve whose peak appears at a greater potential than the two half-wave potentials.

Kies and Nijon (*128*) have described a derivative polarograph which uses a differential galvanometer and the Airey-Smales technique for synchronizing two dropping electrodes. The peak currents obtained were, however, not linear with concentration. Nesvadba (*207*) has described an ingenious method of synchronizing two dropping electrodes by control of the gas pressure over the mercury reservoir and applied this to differential polarography.

Instead of differentiating the polarographic current by means of a condenser circuit, one may obtain a derivative type of curve by superimposing a small alternating current potential upon the current direct voltage sweep and measuring the alternating current component which flows through the cell. If only the microelectrode is polarized, the behavior of the cell is determined essentially by the characteristics of the polarized electrode and the resistance of the cell. The relationship between the amplitude of the alternating voltage and alternating current component can be characterized by an impedance which has its origin in the electrochemical reaction at the mercury electrode. Grahame (*86*) calls this "faradaic impedance" to distinguish it from the impedance corresponding to the double layer capacity. The current measured is the sum of the currents through the faradaic impedance and the double layer capacitor. If the latter component of the current can be made small, as by using very low frequencies, useful differential curves are obtainable. The theory, which is rather involved, has been discussed by a number of authors (*21–23, 44, 77*) and the results of some of these have been summarized in Delahay's book (*43*).

Breyer, Gutman, and Hacobian (*22*) have described simple apparatus using 50- to 60-cycle alternating current and 15-mv. alternating voltage, in which they adjust the direct current potential until the alternating current component is at its maximum, then read the current and applied (direct) potential. The detectability of traces is impressive, $8 \times 10^{-6}N$ zinc being clearly apparent in $10^{-3}N$ cadmium solutions. They observed, as have others, that the alternating current showed a maximum at the half-wave potential and that the alternating current is proportional to concentration at its peak value. Delahay and Adams (*39*) have pointed out that the alternating current component is proportional to the square root of frequency at its peak and that very low frequencies are desirable to reduce the otherwise high capacity current to a manageable value. Reynolds (*228*) has described in detail the construction and testing of an alternating current polarograph. Alternating current polarography has been applied to studies on reversible and irreversible systems (*27*), organic compounds (*20*), and adsorption-desorption processes (*21*). Additional recording instruments have been described (*26, 242*).

An important new approach has been the use of a square-wave alternating voltage superimposed upon the conventional direct current voltage span of the polarograph. A great limitation to ordinary sine-wave alternating current polarography is the very appreciable capacitance current resulting from the double layer. Barker and Jenkins (*11*) have developed an apparatus for superimposing a square wave of small amplitude on the direct current voltage applied to the cell. If the time-constant of the system is small enough, the capacity current falls virtually to zero shortly after each reversal of the voltage. The faradaic current, however, changes slowly because a diffusion process is involved, and as a result, the effect of capacity current can be made negligible by measuring faradaic current just before each change in the applied voltage. By thus eliminating the effect of capacity current, Barker and Jenkins were able to measure concentrations as low as $2 \times 10^{-7}M$ where the electrode reactions were reversible. The theoretical treatment of the square-wave polarograph is exceedingly complex, but the analytical applications look very promising. Ferrett and Milner (*62*) have described some analytical applications of the square-wave polarograph and report that it is possible to measure a component in a 500-fold excess of a more easily reduced substance. Kambara (*121*) has published several papers on the theory and use of the square-wave polarograph.

OSCILLOGRAPHIC POLAROGRAPHY

This field continues to develop along two distinct lines: the first, involving high frequency repeated sweep electrolysis, usually with observation of dV/dt *vs.* V, is championed particularly by Heyrovský and the Czech school of polarographers. Although this technique has found its principal application in the study of the rate and reversibility of electrode reactions, it is also utilizable for analytical purposes. Heyrovský (*97, 98, 100*) has described a portable, commercially available "electronic polaroscope" for use with a streaming or a dropping electrode with controlled drop time, and Kalvoda and Macků (*119, 120*) have described the apparatus in some detail. These authors have developed a technique in which they project alternately, 0.04 second apart, the patterns from two dropping electrodes which are synchronized by mechanical dislodgment of the drops. Quantitative analysis may be done by placing the unknown solution in one cell and a reference supporting electrolyte solution in the other. The constituent to be determined is then added to the latter by means of a buret until the two oscillographic traces superimpose. The authors claim an accuracy of the order of 3%. Details of the apparatus have been described by Kalvoda, Mach, and Doležal (*118*). The properties of the double layer and the effect of adsorption of alcohol have been studied extensively by Heyrovský and Matyáš (*102, 182*) and by Loveland and Elving (*175*). Green (*87*) has modified the Heyrovský-Forejt circuit and uses a double-beam oscilloscope to show $V - t$ and $\frac{dV}{dt} - t$ curves simultaneously. Heyrovský (*99*) has discussed the effects of gelatin, which appears not to hinder diffusion but affects the rates of reactions at the electrode surface. For a general description of this type of oscillographic polarography, the review by Loveland and Elving (*174*) is recommended.

The second approach to oscillographic polarography, often called the "single sweep" method and linked with the name of Randles, utilizes a relatively slow voltage sweep applied once to each individual drop, with the objective of reproducing as closely as possible the conventional type of polarographic pattern. A good introductory discussion is given by Delahay (*39*) in a recent review. Because of the close resemblance of this type of oscilloscopic trace to the conventional polarogram, the method has attracted great interest in practical analysis. Several workers have described apparatus designed to do linear-sweep, cathode-ray polarography (*35, 37, 229*), the latter also providing multisweep traces, and one of particular interest (*251*) is a modification of the Snowden and Page circuit to permit permanent recording with a high-speed galvanometer. Delahay (*42*) and Berzins and Delahay (*14*) have discussed various theoretical aspects of oscillographic polarography, and Papoušek (*216*) has suggested the use of a silver amalgam electrode in oscillographic polarography. Not strictly oscillographic, but nonetheless closely related, is the technique of Kalousek (*116*), in which a commutated alternating current at about 5 cycles per second is applied to the dropping electrode and either the cathodic or anodic branch is measured by the conventional long-period galvanometer. The technique has been applied to the study of adsorption, the reversibility of electrode processes, and identification of reaction products (*117*).

FLOWING SYSTEMS

The use of the dropping electrode and a flowing sample is of considerable importance for application of polarography to continuous analysis. Wilson and Smith (*280*) have studied the effect of cell design and flow rate on apparent diffusion current. After studying vertically ascending, vertically descending, and horizontal flow, they came to the conclusion that descending flow gives the least disturbance. They showed that the normal diffusion current increased smoothly with sample flow up to a given point, whereupon distortion occurred. With constant flow rates, good results are obtainable below the critical disturbance point. Conditions necessary for practical application of this technique are described. Jura has described a shielded dropping electrode for use in flowing systems. Baffles avoid turbulence about the electrode and a period of approximately 2 minutes is necessary for the solution within the shield to come to equilibrium after a twofold change in concentration in the flowing sample (*114*).

Overton and Lewis (*215*) have described an ingenious recording polarograph for use with continuous flow, where variable concentrations of a previously introduced constituent may be encountered. The solution flows through two polarographic cells containing synchronized dropping mercury electrodes, one of which is kept at a potential corresponding to the limiting current prior to the reduction of interest and the other at a potential on the diffusion current of the desired constituent. Larchar and Czuha (*162*) have applied Laitinen's zero current potential method to the automatic recording of oxygen concentration in gases. A cell is described for measuring a flowing sample without turbulence.

NONAQUEOUS SOLVENTS

The use of solvents other than water has ceased to be a novelty in polarography. Many papers on applied problems describe the use of nonaqueous or semiaqueous solvents more or less as a matter of course and reference will not be made to these. A number of authors, however, have studied particular solvent systems in some detail.

Vlček (*266, 267*) has been using concentrated sulfuric acid as a solvent, as has James (*109*), and Laitinen (*177*) has continued his work in liquid ammonia. Schaap, Messner, and Schmidt (*238*) have explored the polarography of a number of metallic ions in ethylenediamine, and Wawzonek and Runner (*276*) have studied inorganic salts in acetonitrile. DeVries and Bruss (*54*) have used anhydrous glycerol, Letaw and Gropp (*165*) and Edsberg, Eichlin, and Gavis (*58*) dimethylformamide, as solvents. Abrahamson and Reynolds (*1*) have examined a number of solvents: methyl ethyl ketone, tetrahydrofuran, acetonitrile, formamide, and pyridine as solvents in the polarography of organosilanes. Radin and DeVries (*223*) mentioned the use of a number of alcohols and mixed solvents for the polarography of aliphatic nitro compounds. Hans and his associates have done extensive preliminary explorations on the use of anhydrous alcohols, glycerol, pyridine, acetic anhydride, and benzene-methanol (*93, 94, 273*).

Arthur and Lyons (*8*) have done a considerable service for beginners in organic solvents polarography by pointing out a number of the difficulties and pitfalls which abound in this type of application. Although nearly everyone who works with organic solvents becomes aware that with many of these compounds it is extremely difficult to remove oxygen completely, that pool potentials may vary radically in the course of a run, that oxygen may react with mercury and give spurious waves, that the high resistance of the solutions leads to difficulty, and that there is danger of reduction at the dropping electrode of oxidation products from the mercury pool, the information is usually obtained by experience and this paper is recommended to anyone who is contemplating starting in this field.

The use of molten salts has continued to attract attention, with both the dropping and solid microelectrodes. Colichman (*30*) has used molten ammonium formate, which has the advantage of melting at only 125° C. and being a good solvent for many salts. Black and DeVries (*16*) have studied lithium chloride, potassium chloride, eutectics as solvents, and Lyalikov (*176*) and Chovnyk (*29*) have studied a number of cations and anions in various fused salt mixtures.

MISCELLANEOUS

The use of controlled-potential electrolysis for purification of supporting electrolytes, a technique in use for some time in a number of laboratories, has been described in detail by Meites (*183*). Proske (*222*) has shown that it is possible to determine water-insoluble substances such as elementary sulfur, peroxidized fats, and anthraquinone by the use of a suitable emulsifying agent to disperse toluene or chloroform solutions of the compound in an aqueous medium. Good polarograms are obtained, although the currents are lower than normal, because of the smaller diffusion coefficient of micelles. Gordon and Jones (*84*) have devised a technique which they term "partition polarography" to permit the determination of organic compounds of similar constitution whose half-wave potentials are too close together to permit separation. The mixture is distributed between two solvents, which are selected so that there will be partially selective extraction of one, and then polarograms are run on the two extracts. With a knowledge of the extraction properties of the system in hand, a determination is possible. They discuss the theory and limitations and give a number of examples. The same technique has been discussed by Dall'aglio (*36*).

Meites (*184*) has shown how it is possible to analyze two-component systems, where the components are not distinguishable polarographically, by measuring the total diffusion current and, in a separate experiment, the total equivalents of the reducible material by means of coulometry at constant potential. An application to the determination of thallium and lead in mixtures is made. Frisque, Meloche, and Shain (*65*) have described a mathematical method for calculating the amounts of two components in a mixture where the waves overlap, giving no clearly defined diffusion current for the first component.

VOLTAMMETRY WITH OTHER ELECTRODES

There exists no general agreement as to what does and does not belong within the scope of polarography. Purists insist that the term applies properly only to the dropping mercury electrode, and some extremists would extend the definition to include techniques as diverse as controlled potential electrolysis and coulometry. For the purposes of this review the essential defining feature of polarography is considered to be electrolysis between a polarizable (usually micro) electrode and an unpolarizable macroelectrode, regardless of form or material of construction.

In voltammetry with microelectrodes, one may have electrodes which are either stationary or in motion, and likewise the solution to be electrolyzed may be stationary or itself in motion. The voltage sweep may be slow (with respect to processes such as diffusion) or rapid. The form of the current-voltage curve will depend greatly on the governing type of mass-transfer process—diffusion or convection–but may be governed also by such things as reaction rates in the solution and rate of electron transfer processes. Consideration of all these factors, taken together in all possible combinations and with varying degrees of importance, leads to a very extensive body of theory: Some idea of the magnitude of it can be gained from a perusal of Delahay's book (*43*). Fortunately, many apparently complicated situations turn out, at least experimentally, to be simple in practice and provide abundant analytical opportunities, whether the theory can be mastered or not. For simplicity, a division has been made here into solid microelectrodes, mercury electrodes other than the dropping electrode, and forced convection electrodes.

SOLID MICROELECTRODES

Many workers have sought to avoid some of the disadvantages of the dropping mercury electrode by the use of solid microelectrodes. With these, very often it is possible to work at much more positive potentials than can be obtained with a mercury electrode; the surface is essentially constant, eliminating the current fluctuations with dropping; there is the possibility of increasing sensitivity by rotation of the electrode or stirring of the solution; and solid electrodes are mechanically stable and resistant to oxidizing agents.

These gains are partly balanced by the disadvantages that there is not a constant renewal of surface, so products deposited on the electrode may change its characteristics; by the fact that the hydrogen overvoltage of a solid electrode is inevitably lower than that of liquid mercury; and that a steady-state diffusion process may take longer to be established at a stationary electrode than at the dropping mercury electrode. Laitinen and Kolthoff (*161*) were the first to obtain accurate and reproducible current-voltage curves with platinum microelectrodes and they showed that the limiting current was governed by a steady-state condition of spontaneous convection about the electrode. Rogers, Miller, Goodrich, and Stehney (*234*) demonstrated that it was possible to obtain automatically recorded polarograms with stationary electrodes, if a sufficiently slow polarization rate was used to get a close approach to the natural convection obtained in point-by-point manual operation. Since then, it has been shown by Nicholson (*209*) that with very rapid voltage sweeps, the limiting current is diffusion-governed, but at intermediate rates both convection and diffusion enter in. Lord and Rogers (*173*) have made a critical comparative study of the use of gold, graphite, and platinum electrodes in reversible and irreversible inorganic and organic reactions. The effects of electrode pretreatment, type of reaction, speed of rotation, and direction of polarization were studied and found to affect both half-wave potential and diffusion current.

Kolthoff and Tanaka (*142*) in an important paper described the study of residual current voltage curves and dissolution patterns for rotating and stationary platinum electrodes in various supporting electrolytes. They have found that on anodic polarization or treatment with oxidizing agents, platinous oxide forms on the rotating or stationary platinum electrode. A clean electrode gives an anodic prewave before oxygen is evolved, for this reason. Anodically polarized or oxidized electrodes show characteristic cathodic dissolution patterns. These observations account for some of the discrepancies between the automatically recorded and manual curves which are frequently observed. Cathodic polarization results in sorbed hydrogen, which may itself in turn produce an anodic dissolution pattern. These factors must be considered and precautions taken to avoid their disturbance in precise polarography with solid electrodes.

Stoll and Berbulk (*256*) have studied rotating solid electrodes of various shapes and treated the limiting currents mathematically. MacNevin and Levitsky (*178*) have suggested the use of platinized-platinum microelectrodes for anodic polarography of organic compounds. If replatinized each time, the electrodes are said to give very reproducible results. Gaylor, Elving, and Conrad (*76*) have used graphite electrodes for anodic polarography of phenolic compounds, and Julian and Ruby have used stationary platinum microelectrodes with a slow rate of polarization for determining half-wave potentials of organic substances (*113*). Sargent, Clifford, and Lemmon (*237*) have used a rotating nickel electrode for polarography in hydrofluoric acid solutions and describe polytrifluorochloroethylene equipment suitable for this purpose. Delimarsky and Abarbarchuk (*53*) have applied solid electrodes to the polarographic determination of metals in nonaqueous solutions, and Armstrong, Heemstra, and Kinchebe (*6*) have described a portable dissolved-oxygen meter based on use of a rotating platinum electrode, which is well adapted to the determination of oxygen in oil-field brines.

Several convenient rotating electrode assemblies for amperometric titration have been described (*4, 95, 240*) and such equipment is now commercially available. Hogge and Kraichman (*106*) have studied the behavior of a rotating-disk electrode at speeds below the forced-convection rate. El Wakkad, Khalafalla, and El Din (*59*) have described a somewhat unusual type of voltammetry with a platinum electrode. They use a plane platinum electrode and electrolyze at a high enough potential to give a limiting current for about 3.5 seconds, during which time the current falls, owing to depletion of material at the surface. They interrupt the electrolysis for a long enough period to restore the concentration of depolarizer at the surface of the electrode to its bulk value and then measure the maximum starting current again. Repeated measurements of the initial limiting current are averaged and the value is found proportional to concentration. This method does not appear attractive for practical work.

Shain and Crittenden (*244*) have explored the use of a Snowden and Page type of cathode-ray polarograph to polarography with the rotating platinum electrode. The results were satisfactory with a sweep of 0.1 to 1.0 volt per second. The limiting factor is the proportion which the capacitative part of the total current contributes to the total. Correction for residual current is possible, if it is not too big compared to the faradaic current. Green (*87*) has used the Heyrovský-Forejt system of oscilloscopic observation with solid electrodes.

Kolthoff and Jordan (*135*) have made ingenious use of the fact that the primary reduction product of oxygen at a solid microelectrode, O_2^-, reacts rapidly with hydrogen peroxide to give hydroxyl ion, oxygen, and hydroxyl radical. At the rotating gold electrode this results in an induced reduction of hydrogen peroxide, so that in the presence of excess hydrogen peroxide the oxygen wave is exalted far above its normal height. With a 10^{-3} M hydrogen peroxide solution, these authors were able to determine oxygen in the range of 10^{-7} M to 5×10^{-6} M. This discovery should prove of great importance in the determination of trace quantities of oxygen.

Attempts have been made to combine the advantages of mercury and solid electrodes by using a solid electrode covered with a film of mercury. Marple and Rogers (*180*) have studied the behavior of a mercury-plated platinum electrode. The hydrogen overvoltage of such an electrode was found to be intermediate between that of a smooth platinum and liquid mercury electrodes. Mercury-plated platinum electrodes gave satisfactory results in quiet and in stirred solutions, but were found to be undependable when rotated. As should be expected, deposition of metals on the mercury-plated electrode results in a change of electrode characteristics. Homogeneous solution reactions, however, do not have this disadvantage and the electrode shows considerable promise, particularly for use at very negative potentials in nonaqueous media, where the dropping electrode begins to stream. Cooke (*33*) has used an amalgamated silver wire electrode in a very similar manner. Typical current voltage curves are given for a variety of reactions. Maxima are not observed and the method is applicable to very dilute solutions. Residual current and the incomplete removal of oxygen, together with depletion of the solution, are factors which limit the application to very dilute solutions. Cooke found his electrodes to retain their calibration characteristics, but Rogers (*180*) has reported both difficulty in preparing electrodes and a tendency to change calibration with time.

Instead of rotating the electrode or stirring the solution, it is also possible to avoid spontaneous convection effects and bring a larger volume of solution in contact with the electrode by setting the electrode into rapid vibration. Although little fundamental work has been done with vibrated electrodes, it is clear that limiting currents are dependent upon intensity of vibration up to a certain point, after which the limiting current is controlled by forced convection (*168*). Vibrating electrode systems have been described by Lindsey (*167, 168*), Roberts and Meek (*233*), and Dirscherl and Otto (*56, 57*). The chief application would seem to be in amperometric titrations.

MERCURY ELECTRODES

Mercury electrodes in almost every conceivable physical form have been tried for polarography. The streaming electrode of Heyrovský and Forejt (*101*), widely used in oscillographic polarography despite inherent mechanical difficulties, has been studied by a number of workers. Valenta (*265*) has shown that discontinuities in the charging current can be traced to adhesion of the solution in the vicinity of the electrocapillary zero. Koryta (*144*) used a freely falling downward stream to avoid breaking the diffusion layer. Diffusion and kinetic currents for reversible and irreversible systems were studied. Weaver and Parry (*277*) developed an equation for the limiting current at the streaming electrode and subjected it to experimental verification. Lévêque (*166*) has described an ingenious streaming electrode in which a film of mercury falls down an amalgamated platinum wire 0.5 mm. in diameter and 0.8 to 2.5 cm. in length. The stream terminates in a pool of diethyl phthalate, so that the length of the stream is fixed and reproducible. This type of streaming electrode offers a very constant surface and does not use as much mercury as the conventional design. The limiting currents observed with this electrode are about 30 times those with the dropping mercury electrode, but of course the charging current is correspondingly increased also.

Lee (*163*) described the use of a rotating mercury electrode in which a cup with an annular groove holding approximately 0.1 ml. of mercury was rotated at 1200 r.p.m. with a radius of about 1 cm. Current-voltage curves were measured with conventional polarographic equipment. Well defined waves were obtained with a sensitivity comparable to the rotating platinum electrode, while retaining the favorable hydrogen overvoltage characteristics of the dropping mercury electrode. Diffusion current was shown to be proportional to concentration for metal ions in the range 10^{-4} to $10^{-7}M$. The range of potentials is more limited than with the dropping electrode, as the supporting electrolyte is deposited more readily than with the dropping electrode. The theory of this type of electrode was developed in the same paper. Although the rotating mercury electrode would appear to have considerable promise, it does not seem to have been adopted by other workers.

Berman, Saunders, and Winzler (*12*) have suggested the use of a very rapidly vibrated dropping mercury electrode for polarography in agitated solutions. The electrode is vibrated by an arm actuated by a wheel rotating at 4860 r.p.m. with an eccentricity of 0.012 inch. Although no fundamental study was made of the properties of this electrode, it was observed that the dislodgment of drops was too rapid to permit individual drop traces to be seen and that normal polarograms with a reasonable linearity of current with concentration were obtained. Kolthoff (*141*) has very recently developed a rotated dropping mercury electrode. The tip of a conventional dropping electrode is bent so that the mercury is flowing in a horizontal direction as the drop forms and the electrode is rotated at 200 to 300 r.p.m. The result is a stream of exceedingly fine drops. The sensitivity is about 10 times that of the conventional dropping electrode and it appears suitable for trace analysis and amperometric titrations. Interesting stirring effects at the surface of the mercury are caused by the rapid flow, and no constancy of limiting current with concentration is obtained unless maximum suppressors are present or the ionic strength is comparatively high. Good results are obtained in the presence of gelatin and the electrode shows great promise.

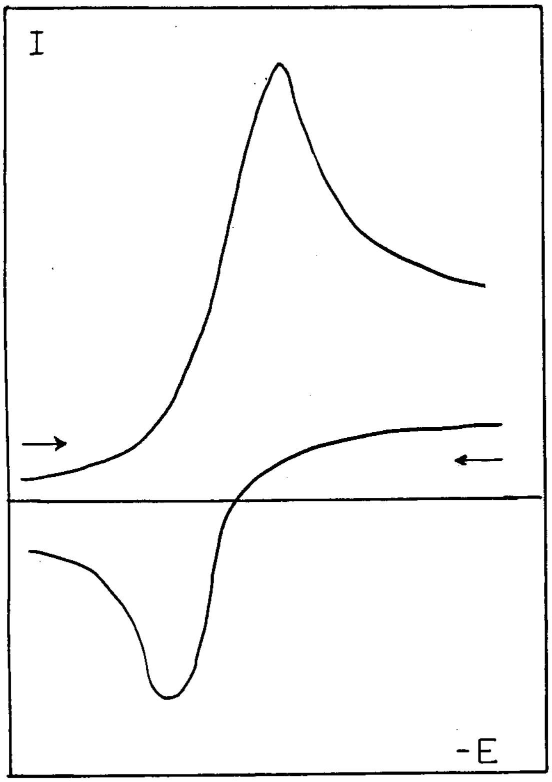

Figure 1. Forward and reverse polarograms of a reversibly reduced substance at a quiet mercury pool—e.g., 1,6-anthraquinone disulfonate

Anodic wave on reversal is due to reoxidation of material reduced in the cathodic portion of the wave. Both curves show the characteristic "time maxima" due to depletion of the reacting material at the electrode

One of the factors limiting the sensitivity of the ordinary dropping electrode is the small surface area of the drop. Streuli and Cooke (*259, 261*) have sought to circumvent this difficulty by studying the polarographic characteristics of quiet mercury pool electrodes. Using a pool with an area of about 3 sq. cm. and conventional polarographic equipment for obtaining current-voltage curves, they observed the current-voltage characteristics of a number of metal ions being reduced to amalgams and, in a later paper (*260*), organic compounds. A reducible substance characteristically gives a high initial peak which falls off, owing to depletion of material at the surface of the electrode. The shape of the curve is a function of the number of electrons and the polarization rate. Peak heights are linear with concentration and were found to be reproducible to 4 to 7%.

A reverse scan usually gives an anodic current due to reoxidation of the material deposited at or on the electrode (Figure 1). The situation pertaining in this type of electrolysis is entirely analogous (except for the time scale) to single-sweep oscillographic polarography, and qualitative agreement is found with the equations of oscillographic polarography. In the applications to organic polarography it was observed that although results were in many respects similar to those obtained with the dropping electrode, certain striking differences sometimes occurred. Benzil, for example, which gives a single wave at the dropping electrode, gives a double wave at the pool, while nitrobenzene showing two waves at the dropping electrode shows a single step at the pool. Hydrogen discharge comes 0.2 to 0.3 volt earlier at the pool than at the dropping electrode.

More recently, Rosie and Cooke (*235*) have used the mercury pool cathode with stirred solutions. A stirrer is positioned above the pool and it is found that the limiting current is linear, approximately, with speed up to about 1200 r.p.m. The currents are very sensitive to stirrer position if it is close to the electrode, but at 5- or 6-mm. distance, the currents are stable and a 1-mm. difference in distance amounts only to about 3% error. The sensitivity of this arrangement is about 300 times that of the dropping electrode and the shape of the curve was found to be independent of scanning rate. With the electrolysis of small volumes of dilute solutions with relatively large electrodes, however, depletion of material is appreciable and it was observed the bulk concentration of the solution dropped about 20% in 10 minutes. Rosie and Cooke studied a number of systems: ion-metal, ion-amalgam, ion-ion, and ion-molecule, and this type of polarography appears to be worthy of serious consideration in practical problems.

Arthur and coworkers (*7, 9*) have described the use of a stationary mercury-drop electrode in a stirred solution. The electrode is formed from a drop at the end of a wax-coated tube held vertically and a bell-shaped glass tube of larger diameter is suspended over the electrode and rotated at about 600 r.p.m. This type of stirring appears to be exceptionally uniform and good polarograms are obtained. Diffusion currents are large and the residual current is considerably less than that observed at the dropping mercury electrode. Marple and Rogers have applied the same type of stirring to solid electrodes with good success (*180*). Kahle and Reif (*115*) have described a somewhat similar stationary mercury electrode, but with a different method for putting the solution in motion. A nitrogen stream acting as an air lift carries the solution up an arm and feeds it into a reservoir above the electrode, from which it flows past the electrode and back to the inlet of the pump. Good polarograms were claimed, with diffusion currents proportional to concentration and currents of the order of tenfold greater than those obtained with the dropping mercury electrodes. Yashiro (*281*) has described a pinhole electrode which allows a single stationary drop to come in contact with the solution. As would be expected, the residual current is low and the sensitivity comparable with that of the dropping electrode. Simonnin and Quintin (*245*) have described a stationary mercury dropping electrode with a renewing surface. A column of mercury in a tube similar to the electrode of Arthur is renewed by drops falling from above.

FORCED CONVECTION ELECTRODES

When a solution moves past an electrode surface, the only effect of increased flow rate for rapid reactions is normally an increased current due to effective increase in the diffusion rate. When the rate of flow becomes extremely great, diffusion is no longer the governing factor and the limiting current is dependent upon the convective transfer of material to the electrode surface. Kolthoff and Jordan (*136, 137*) have recently described convection-governed electrode systems and demonstrated great analytical advantages in their use. A stationary platinum electrode in a very rapidly stirred solution shows limiting currents which are not diffusion-controlled and which are independent of the viscosity of the medium over a wide range. The current is governed by the simple relation

$$i = nFA\tau C$$

where τ, the convection coefficient, is the volume of solution in microliters per second per square centimeter of electrode area and depends upon prevailing hydrodynamic conditions. Since the diffusion coefficient of the reducible substance does not enter into the equation, the same calibration factor applies to all substances which undergo rapid and reversible reduction if they have the same value of n. Convection currents vary with temperature by about 2% per degree Centigrade.

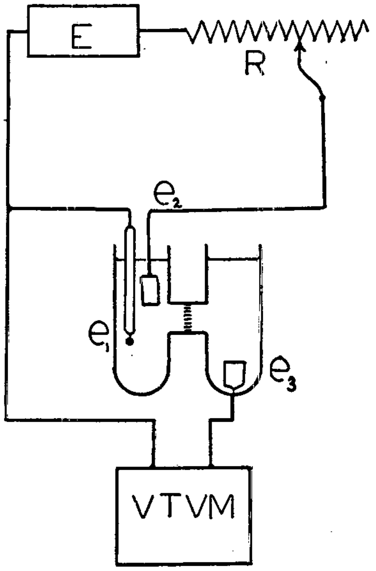

Figure 2. Basic circuitry for potentiometric polarography

E. Constant voltage source (150 volts)
R. 90-megohm variable resistance to provide current scan
e_1, e_2, e_3. Cathode, working anode, and reference electrodes, respectively

Kolthoff, Jordan, and Prager (*139*) have studied the convection-controlled mercury electrode. A tube 1.5 to 2.0 mm. in inside diameter is held vertically with a column of mercury in it which is just level with the upper end of the tube. A large, multibladed stirrer is positioned 5 to 8 ml. above the electrode and rotated at a speed between 800 and 1200 r.p.m. The electrode is quiescent in spite of the intense stirring, and half-wave potentials were observed to be constant and independent of both concentration and rate of polarization. The sensitivity is about 20 times greater than that of the dropping mercury electrode. Curves of current-voltage, current-time, and voltage-time at constant current were determined. In addition to its obvious applications in polarography, the convection-controlled mercury electrode is useful for determining diffusion coefficients of metals in amalgams.

More recently Kolthoff and Jordan (*138*) have studied anodic halide waves at the convection mercury electrode. Convection electrodes will undoubtedly find great application both in conventional polarography and in amperometric titrations.

POTENTIOMETRIC POLAROGRAPHY

Adams, Reilley, and Furman (*3*) have described a most ingenious reversal of the conventional polarographic technique, in which current is made the independent variable and the resulting potential is measured (Figure 2). This method, called "potentiometric polarography" or "controlled current scanning polarography," has the advantage of manipulative simplicity and is particularly well adapted to work with solid electrodes. The apparatus consists of a variable source of constant current and a vacuum-tube voltmeter or the equivalent for measuring the potential of the microelectrode with respect to a reference half-cell. Adams, Reilley, and Furman used radio B batteries in series with a 10,000-ohm to 19-megohm resistor as the current source, and a pH meter for measurement of the cell potential. Current-voltage curves determined by this method and by conventional polarography were essentially identical for trivalent iron in hydrochloric acid at a rotating platinum electrode. One advantage of current-scanning over voltage-scanning with platinum electrode polarography in quiet solutions is that diffusion currents are smooth and do not show the usual peak of "time maximum." The technique has been recommended especially for exploratory work in coulometric analysis. A simple procedure for the estimation of iron in iron ore mixtures was developed, which gave consistent results within 1% of the true

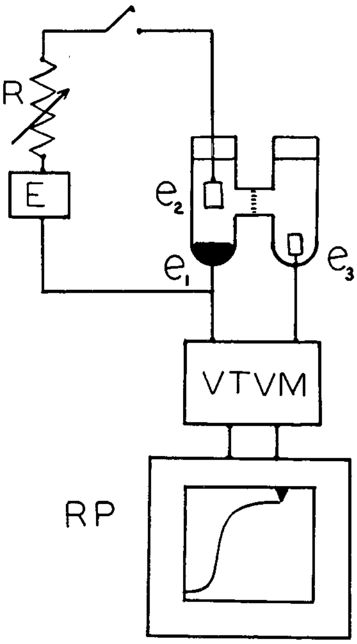

Figure 3. Basic circuitry for chronopotentiometry

E. Constant voltage source—e.g., 100 volts
R. Current governing resistor—e.g., 10^7 ohms
e_1. Mercury pool cathode
e_2. Working anode
e_3. Standard reference electrode
VTVM. Vacuum-tube voltmeter
RP. Strip-chart recording potentiometer

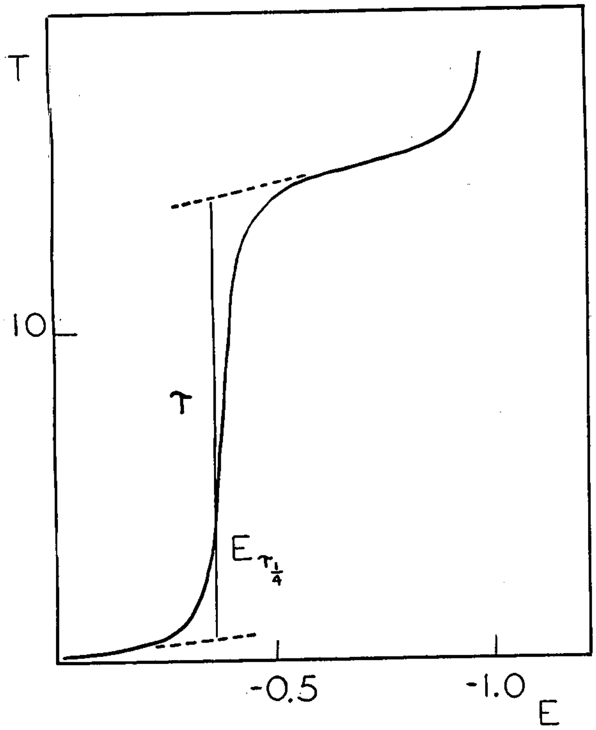

Figure 4. Typical chronopotentiogram of lead in nitrate medium

Plot of E of cathode (measured against S.C.E.) as a function of time. Time constant, τ, here about 13 seconds, is proportional to concentrations for a given set of experimental conditions, and the potential $E_{\tau_{1/4}}$ is characteristic of the substance reduced and the medium

iron content. Adams (*2*) using an experimental technique very similar to that developed by Gauguin and coworkers (*13*, *71*, *73*, *74*) for other purposes has shown the application of current-scan polarography to titration. This technique is the current-scan polarographic analog of amperometric titrations. Titrations of zinc with ferrocyanide, arsenic with bromine, iron with cerium, and cadmium with ethylenediaminetetraacetic acid were described.

CHRONOPOTENTIOMETRY

The idea of measuring voltage-time curves at an electrode when a constant current is forced to pass through the system is not a new one, having been used by a number of investigators around the turn of the century for the purpose of studying diffusion processes. It is only recently, however, that the potentialities of such a method in analytical chemistry were first realized (*78*). The theory of this type of electrolytic process has been rather fully developed by Delahay and his associates (*45*, *47*, *49*) and the results have been summarized in a recent review by Delahay and Mamantov (*46*).

A typical circuit for chronopotentiometry is shown in Figure 3. When a constant current is forced through an electrolysis cell and the potential of the electrode is measured against a reference half-cell, it is found that for a typical system involving a depolarizer in the presence of a supporting electrolyte, the potential rises rapidly to the decomposition potential of the depolarizer. It then rises comparatively slowly until the depolarizer is depleted at the electrode surface, whereupon it raises rapidly again (Figure 4). The essence of the method lies in measurement of time necessary for a state of concentration polarization to be established. For an electrode process without kinetic complication, the square root of this time, commonly called the "transition time," is proportional to the concentration of the depolarizer and inversely proportional to the current density. By appropriate choice of concentrations and current densities it is possible to obtain transition times in a conveniently measurable range. Reilley, Everett, and Johns (*226*) have made an extensive experimental evaluation of the technique and report excellent results for many systems. The quiet mercury pool is particularly well adapted to chronopotentiometry because of its constant and reproducible surface and its high hydrogen overvoltage.

The charging time component of the current varies inversely with the current density, whereas the transition time for diffusion-controlled electrochemical reactions varies inversely with the square of the current density. This means that halving the current density doubles the charging time but increases the transition time fourfold. Thus the use of small current densities necessary for very low concentrations actually produces an enhancement of the ratio of faradaic to condenser current and yields a greater over-all sensitivity. A fundamental limitation to increased sensitivity is that transition times greater than a minute or so become difficult to measure accurately because of instability of the diffusion layer. When two constituents are determined from the same curve, the concentration of the first to be reduced may be calculated by its transition time in the usual way. This is not true of subsequently reduced constituents, as the total electrolytic current is divided between the diffusion currents. The state of concentration polarization with respect to the second ion is reached less rapidly and hence the transition time is correspondingly increased. A consequence of this enhancement is the possibility of increasing the detectability of one ion by addition of another more easily reduced.

Nicholson and Karchmer (*210*) described a versatile pen-recording instrument for making chronopotentiometric measurements and gave the results of a study of lead in nitrate medium. With this system they recorded transition times reproducible to 1 or 2% over a range of 3 to 10 seconds and a constancy of $i\tau^{1/2}/C$ to about 1% in the range of 2×10^{-4} to $2 \times 10^{-2}M$. The $E_{\tau/4}$, which is the chronopotentiometric analog of $E_{1/2}$ in ordinary polarography, was nearly constant to about 30 mv. over most of the concentration range examined and had the same order of magnitude as the polarographic half-wave potential in the same medium.

Gierst and Mechelynck (*79*) have described apparatus for constant-current measurements of a similar sort. Reilley and Scribner (*227*) have used chronopotentiometric measurements to follow the course of titrations. This is the chronopotentiometric analog of amperometric titrations. Reilley, Cook, and Furman (*224*) have described a variety of polarized electrode potentiometric titrations which they call "derivative polarographic titration."

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Organic Polarography

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THE past 2 years since the last review have seen a continuation of the great interest in organic polarography and the publication of a large number of papers. As in the past, some of these studies have duplicated or reinvestigated previous work. In this review attention is not called to such duplications, unless the results are in disagreement with those already in the literature.

Developments in this period which are noteworthy, because they help extend the field of polarography, are the greater use of anhydrous solvents, applications of the Hammett equation to polarographic data, and the combining of special techniques of separating organic compounds with the polarographic method in the analysis of mixtures of compounds.

In the use of anhydrous solvents in this field preliminary data are reported for an ammonia–ammonium nitrate mixture (*248*), hydrazine (*115*), ethylenediamine (*369*), and liquid hydrogen fluoride (*360*). Aromatic carbonyl compounds show a normal behavior in formamide (*250*) and in mixtures of formamide and acetamide (*165*). The deposition of hydrogen ion in acetonitrile (*433*) and ethyl alcohol (*434*) has been found to occur at more positive potentials than in water. The reduction of aromatic hydrocarbons in dimethylformamide (*448*) and of quinones in acetic acid (*415*) will be discussed later. For a reference for comparative studies in different solvents potassium ions have been suggested (*432*).

The use of mixtures of alcohol and water continues to be common. The influence of the viscosity in such combinations in lowering the wave height is again pointed out (*174*). A similar effect on the current is noted at the polarographic micelle point, when sodium benzenesulfonates are used to solubilize nitrobenzene (*168*).

Applications of the Hammett equation to half-wave potentials for groups of similar compounds have been numerous (*176*, *476*, *484*). The significance of such half-wave potential plots has been questioned for irreversible systems (*24*). The good agreement obtained in most cases suggests that perhaps in the electrode reaction

$$O + ne \rightleftharpoons Z \xrightarrow{k} R$$

the kinetics of the irreversible second step are the same for each member of the series.

The combining of polarographic techniques with chromatography (*199*), paper chromatography, and paper electrophoresis has made possible analyses of mixtures of organic compounds which would ordinarily interfere with each other in a direct polarographic analysis.

In studies of solid electrodes graphite has been found to be a better electrode for organic oxidations than gold or platinum (*258*), the construction of a cathode-ray polarograph for use with a rotating platinum electrode is described (*381*), and the use of two cells with identical electrodes is suggested for better reproducibility with rotating platinum electrodes (*80*).

Oscillographic polarography has seen the application of a new electronic polarograph to pharmaceutical analysis (*143*), the development of stabilized polarograms using the dropping mercury electrode (*192*), and the use of the technique in studying the mechanism of reduction of various organic compounds (*412*).

A polarized mercury pool electrode can successfully replace the dropping mercury electrode (*401*) with most organic compounds and gives a behavior similar to that observed in oscillographic work (*399*). The sensitivity is greater than that for the dropping mercury electrode, but the range of potential available is less; no wave, for example, is obtained for anthracene. The concentration range can be extended further by using stirred solutions (*343*).

The important features of organic alternating current polarography have been summarized (*36*) and applications to chloranilic acid and methylene blue described (*38*).

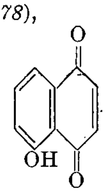
In a unique application of polarography a relationship between organic corrosion inhibitors and polarographic maxima suppressors is reported (*121*).

For the evaluation of electrode reactions a microcoulometric method (*337*) and a controlled cathode potential apparatus (*198*) are described.

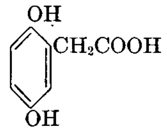
Specialized reviews of interest cover the applications of polarography, oscillographic polarography, and polarographic titrations to pharmacy (*147*, *193*, *441*, *485*), newer developments in polarography (*374*), kinetic currents (*32*), the evaluation of rate constants of reactions involved in electrode processes (*33*), and the influence of surface active compounds (*146*, *483*) and of structure (*477*) on the polarography of organic compounds.

REVERSIBLE SYSTEMS

In fundamental work the polarographic method determined the oxidation-reduction potential of juglone (I) in aqueous media (*478*),



I



II

indicated that anthraquinone (*172*), 1,2-benzanthraquinone, and 1,2,5,6-dibenzanthraquinone are reversible systems in glacial acetic acid (*415*), found the oxidation wave for hydroquinone and thiobarbiturates to be affected in phosphate buffers by film formation (*481*), and compared the effect of substituents on the half-wave potentials of various anthraquinones and other classes of dyes (*16*).

The method has also been used to determine juglone and ascorbic acid in walnuts and in various parts of the walnut tree (*478*), hydroquinone in various monomers (*99*) and in urine after oxidation with ceric salts (*17*), gentisic acid (II) after separation from hydroquinone chromatographically (*234*) in urine (*81*), and naphthoquinone in phthalic anhydride (*9*).

The measurement of the diffusion coefficient of benzoquinone in solutions of potassium salts is reported (*397*).

The reduction of chloranilic acid has been used as the basis for the indirect determination of calcium (*39*).

In the *o*-quinone (*o*-benzoquinone) system gallic acid and protocatechuic acid show an adsorption and a diffusion wave (*436*). The wave for gallic acid at a platinum electrode can be employed in the determination of this compound in oak bark (*393*).

The reduction of iodoadrenochrome and iodonoradrenochrome, obtained by the iodate oxidation of adrenaline and noradrenaline, serves as a method for the indirect determination of these two amines (*140*). On the basis of polarographic data presented previously, adrenochrome is believed to function in organisms as a hydrogen transmitter (*472*).

Compounds of other reversible systems such as the phenazines, oxazines, and thiazines have been found in small concentrations to catalyze the evolution of hydrogen on the dropping mercury electrode (*439*). The mechanism of reduction of several of these has been studied using special techniques (*191, 408, 411*).

The method proved successful in following the photochemical reduction of riboflavin (*284*) and the formation of deuteroflavines (*215*) required less time for assaying riboflavin in pharmaceutical preparations than the fluorometric method (*378*), and can determine the properties of vat dyes (*175*).

The azobenzene-hydrazobenzene system has been found to be reversible thermodynamically at the dropping mercury electrode in the buffer range of pH 2 to 6. At higher pH values hydrazobenzene is oxidized at values which are about 0.07 volt more positive than those obtained for *trans*-azobenzene (*449*). In acid medium azobenzene gives a second reduction wave corresponding to the formation of aniline (*67, 449*).

In other studies on azo compounds reductions are reported for Evans blue (T-1824), for the same dye in the presence of plasma (*272*), for the food coloring dye amaranth (*263*), for the azoproteins made by coupling diazonium salts with egg albumin (*40*), and for methyl orange in the presence of albumin (*37*).

The method has also shown that *cis*-azobenzene is not an equimolar mixture of azoxybenzene and hydrazobenzene but a true geometrical isomer (*450*), that the effect of acids on the reduction of azobenzene can be used to study the kinetics of the dissociation of acids (*74, 353*), and that the wave for the aluminum complex of Pontachrome Violet S. W. (sodium salt of 5-sulfo-2-hydroxybenzeneazo-2-naphthol) is decreased by fluoride ion and can be used for the determination of this ion (*264*).

IRREVERSIBLE SYSTEMS

ALCOHOLS AND GLYCOLS

Even though the oxidation of ethyl alcohol occurs at the platinum electrode (*31*), the more common method used for this compound is an indirect one in which the oxidation is carried out chemically with potassium dichromate and the excess dichromate is determined polarographically (*292, 334*).

The marked effect shown by glycerol and sorbitol on the boric acid wave has not yet been exploited quantitatively (*240*).

ALDEHYDES

Fundamental work on the reduction of formaldehyde has continued and results have been reported in unbuffered solutions (*34*), at a mercury jet electrode (*260*), and in solutions at low temperatures containing either ethyl alcohol or heavy water (*380*). In the last investigation a second wave was reported which was diffusion-controlled. The actual reduction process involved for this step is not known.

Formaldehyde has been found to reduce at more positive potentials than normal in a solution containing lithium chloride and aluminum salts. The formation and involvement of lithium aluminum hydride have been proposed as an explanation for this behavior (*144*).

In the quantitative determination of aliphatic aldehydes the method has been found best for determining aldehydes in ether (*218*) and has been used to follow the formation of formaldehyde in the pyrolysis of trimethylene oxide (*27*) and acetaldehyde in the hydration of acetylene (*376*). The method has determined the formaldehyde and acetaldehyde resulting from the cleavage of glycols obtained from isomeric octenes (*149*) and, based on the determination of acetaldehyde, has served as a measure of glycolysis (*78*).

Indirectly aldehydes can be determined by precipitating with 2,4-dinitrophenylhydrazine and determining the unchanged reagent polarographically (*322*).

The reduction of semicarbazones of aliphatic aldehydes and ketones indicated that the stability constants are greater for the aldehydes (*395*).

In the sugar series investigations have been varied. The most important of these is the study of the polarographic behavior of α-glucose with time. The results point to the presence of about 0.003% of the free aldehyde in the equilibrium mixture. The observation that the limiting current for α-glucose was higher than that observed for the equilibrium mixture (*259*) has interesting implications and is worthy of further study.

The rates of the ring-aldehyde transformation previously reported have been found in error (*35*) and parallel the rates of reaction between various sugars and ethyl acetoacetate (*342*).

The polarographic technique has been combined with paper chromatography to follow the action of alkali on glyceraldehyde (*333*), and with the conductometric determination of ash to evaluate the quality of sugars (*46*).

Neomycin, a streptomycin antibiotic, has no polarographically reducible group (*320*).

Investigations on unsaturated aldehydes have used the method to determine crotonaldehyde in vinyl acetate (*467*), and to follow the cyclization of citral with acid, its cleavage with alkali, and condensation with acetone (*15, 375*). The results obtained for citronellal and total aldehydes in essential oils have been low (*138*).

Energies calculated by the molecular orbital method for the conversion of polyene aldehydes to free radicals lead to half-wave potentials which are in fair agreement with the experimental values (*377*).

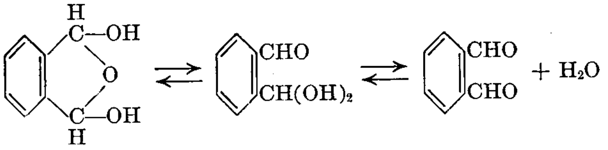
Conditions for the quantitative determination of glyoxal are given (*89*) and the differences between the behavior of aliphatic and aromatic aldehydes are presented (*102*).

Work on aromatic aldehydes has seen a reinvestigation of benzaldehyde in the pH range 1.1 to 13 (*156*). The interpretation for the decrease of the second wave with pH has ignored the work of Ashworth (*6*) on this subject. The suggestion that benzaldehyde is reduced to benzyl alcohol in acid medium is contrary to the results reported previously (*317*), that only hydrobenzoin was isolated in a large-scale electrolysis of benzaldehyde at pH 1.3.

A similar study of 39 other benzaldehydes has been carried out to determine the effect of substituents on the reduction of the aldehyde group (*157*). The half-wave potentials at pH 13 give a good Hammett plot (*129*).

Values are also reported for the reduction of the naphthaldehydes, 9-anthraldehyde, and 9-phenanthraldehyde, and theoretical calculations have been made involving the half-wave potentials (*124, 371*).

The reduction of phthalaldehyde in buffers of pH 3 to 14 indicates that this compound is hydrated in solution (*116*). Since only a monohydrate has been isolated, the structure must be cyclic and the polarographic behavior must be dependent on the following transformations:



The polarographic method has been used to determine benzaldehyde in *l*-phenylacetylcarbinol produced by fermentation

(*290*) and 2,4-disulfobenzaldehyde and *p*-aminobenzaldehyde in mixtures with *p*-nitrotoluene (*429*), and to follow the kinetics of the reaction of aromatic aldehydes with cyanide ion (*488*).

Data are also given for the reduction of *syn*- and *anti*-benzaldoxime (*423*).

KETONES

Most of the work with aliphatic ketones has dealt with the reduction of their condensation products with nitrogen compounds. Results, for example, are given for the reduction of cyclopentanone, cyclohexanone, and methylcyclohexanone in the presence of ammonia, primary amines, and amino acids (*42*). Amines are unsuitable for determining 17-keto steroids, but Girard reagent D, $(CH_3)_2NCH_2CONHNH_2$, proved satisfactory in this respect (*41*).

Comparisons of the half-wave potentials for the Girard reagent T, $(CH_3)_3NCH_2CONHNH_2$, derivatives of 24 aliphatic ketones have indicated that the steric hindrance of branched alkyl groups has an effect on the reduction (*468*).

Results are also reported for π-oxocamphor and its derivatives (*5*).

The method has been used to follow the oxidation of cyclohexanone (*100*) and the hydrolysis of cyclohexanone oxime (*437*).

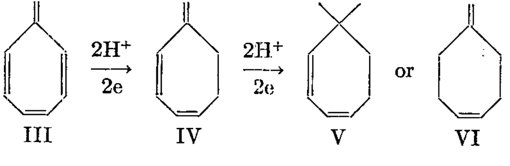
In the diketone series the irreversibility of the reduction of diacetyl has been confirmed oscillographically (*327*), and the 2-electron reduction of camphorquinone has been verified (*249*).

Studies are reported on the reduction of dimedone (*314*) and cyclohexandione dioxime (*53, 170*), and on the use of the method to investigate complex formation between fructose and germanium dioxide (*356*).

In the reduction of α,β-unsaturated steroids a second wave caused by dissociation is reported in alkaline solution (*489*). For their determination in associated colloids, extraction with benzene or petroleum ether is required before the analysis can be performed (*86*). Similarly, fractionation of corticosteroids through paper chromatography of their 2,4-dinitrophenylhydrazones is necessary before the analysis (*75*) is carried out.

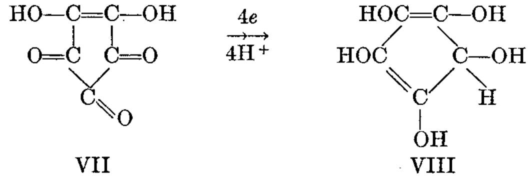
Among other natural products having this structure, pyrethrins are more accurately determined at the dropping mercury electrode than by any other method (*306*). The technique, however, cannot distinguish between the two isomers (*305*). Terramycin, which gives suitable waves for quantitative work (*235*), can be distinguished qualitatively from aureomycin at pH 6.5 but cannot be determined quantitatively in the presence of this compound (*420*).

In the tropone and tropolone series, which has been summarized recently (*318*), a number of new examples have been investigated. Tropone (III) is reported to give two waves of 2 electrons each and to form the alcohol (V) (*11*). The possibility that ketone VI was formed instead by the reduction of the α,β-unsaturated ketone IV was not eliminated.



Reports are also given on the reduction of β-hydroxytropone (*178*) and hinokitol (α-isopropyltropolone) (*256*). The former resembles tropone (III) in its behavior, whereas the latter can be titrated with copper sulfate.

In other work large-scale reductions have indicated that dimerization of α,β-unsaturated ketones produces dimeric ketones rather than the unsaturated pinacols (*226*), and that the reduction of croconic acid (VII) probably goes stepwise to the pentahydroxy compound (VIII) (*3*).



The aromatic ketone series has seen the investigation of various acetophenones (*50*), their oximes (*51*), benzophenones (*52*), methyl naphthyl ketones (*47*), and thiobenzophenones (*361*). In the last class one 2-electron wave is formed and must give a mercaptan as the product. A nitro group, if present in the *p*-position, is reduced only to the hydroxylamine, whereas the corresponding benzophenone derivative forms *p*-aminobenzohydrol (*362*).

$$(C_6H_5)_2C{=}S + 2e + 2H^+ \rightarrow (C_6H_5)_2CHSH$$

The mechanism of the reduction of carbonyl compounds has been studied oscillographically (*410*) and the reduction products formed from benzophenone in the polarographic cell in a sodium chloride solution have been studied with ultraviolet spectra (*370*). The claim that benzopinacolone is formed under these conditions is unusual, as this compound results from the reduction product, benzopinacol, only in the presence of acids.

The half-wave potentials for the reduction of imines of several alkyl phenyl ketones have been found to parallel the relative catalytic reduction rates (*323*), whereas the values for various benzophenones, when plotted against the corresponding infrared frequencies, give a straight line (*118*).

The Hammett equation can be applied to the half-wave potentials of various benzophenones, acetophenones, and benzaldehydes, with the exception of the *p*-phenyl derivatives (*363*).

The use of polarographic behavior in the determination of carbonyl compounds has been summarized (*332*).

ACIDS AND THEIR DERIVATIVES

The reductions of carbon dioxide and of oxalic acid have received some attention because of the uncertainty of the electrode reactions. The wave for carbon dioxide has been ascribed both to the reduction to formic acid (*419*) and to the discharge of hydrogen ions (*435*). The direct reduction of the compound, which seems the least likely, is a possibility that has to be considered, as carbon dioxide gives a reduction wave in anhydrous dimethylformamide (*448*).

Oxalic acid is reported to give a wave in acidic buffers and to form a product that will give an anodic wave (*241*).

Hydroxy acids in the aliphatic series, which are not reducible, have been determined indirectly. The method for citric and isocitric acids is dependent on the conversion to and the polarographic reduction of pentabromoacetone (*96*), and that for mandelic acid is based on its oxidation with zinc permanganite to benzaldehyde (*26*).

In the aromatic series salicylic acid, its methyl ester, and benzoic acid give a 2-electron wave in alkaline medium (*134*). Reduction is also reported for dimethyl terephthalate (*309*) and dialkyl phthalates (*453*). Large-scale electrolysis data indicate that the former is reduced to *p*-carbomethoxybenzyl alcohol and the latter to phthalide. Further reduction of phthalide gave an unidentifiable product with aldehydic properties.

The method has been used quantitatively to determine phthalic anhydride in alkyl resins after saponification of the polymer (*120*).

In the α-keto acid series the polarographic results for pyruvic acid and for phenylglyoxylic acid have been used extensively to test equations derived for kinetic currents (*8, 137, 141, 227, 232, 456, 457*).

Other investigations have determined pyruvic acid in lactic acid and calcium lactate (*482*); pyruvic acid, oxalacetic acid, and α-ketoglutaric acid as the 2,4-dinitrophenylhydrazones after separation by paper electrophoresis (*296*); α-ketoglutaric acid directly (*311*); and opianic acid in alkaloids (*167*).

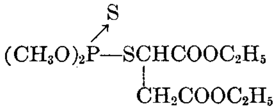
The kinetics of the reaction between ammonia and pyruvic acid have been studied oscillographically (*101*).

Ascorbic acid, which gives an irreversible anodic wave, has been used to check expressions derived for electrode reactions involving a product which is removed by an irreversible step (*207*, *231*).

The determination of this compound has been applied quantitatively to foods (*358*), lemon juice (*382*), vegetables (*487*), and etiolated seedlings (*127*), and has been used to follow its autooxidation (*77*).

In the unsaturated acid series reductions have been reported for acrylic acid esters but not for vinyl ethers (*29*), for acrylic acid esters in a methanol-benzene mixture as a solvent (*4*), itaconic acid (*271*, *297*), acetylenedicarboxylic acid (*269*), muconic acids (*104*), and ethyl 1,1,2,2,3,4-butene-3-hexacarboxylate (*310*).

The polarographic method for fumaric acid and maleic acid, which required precise pH control (*94*), has been used to determine fumaric acid in fermentation samples (*247*) and maleate unsaturation in polyesters (*150*), has proved that maleic anhydride is isomerized to fumaric acid in polyester formation (*105*), and has followed the catalytic hydrogenation of maleic-fumaric acid and fumaric-oleic acid mixtures (*270*). This method is also the basis for the indirect determination of Malathione (IX) (*188*) and malic acid (*131*). Both compounds are converted into fumaric acid by alkali.



$$(CH_3O)_2\overset{S \nearrow}{P}—\underset{CH_2COOC_2H_5}{SCHCOOC_2H_5}$$

IX

The results for these two acids have been used to check a relation derived for the polarographic reduction of dibasic acids (*230*).

Reduction occurs for nitrates of glycols (*452*) and is the basis for the analysis of nitroglycerin in double-base powder (*451*).

The lower melting isomer of hydroxyurea is reported to give a wave corresponding to a reduction to urea (*217*).

ORGANIC HALOGEN COMPOUNDS

In studies of halogenated hydrocarbons the polarographic method has been applied in a variety of ways. The reduction of carbon tetrachloride has been found to be markedly affected by solvents and supporting electrolytes (*261*). The amount of liquid-liquid junction potential in some of these differences was not evaluated.

Studies are also reported on triphenylmethyl chloride (*243*) in various solvents, and on allyl bromide (*210*) and allyl bromides substituted with methyl, bromine, and chlorine in the α- and β-positions (*211*). The prewave noticed for the allylic compounds may be a peroxide wave.

This method has been applied to the determination of unsaturated organic compounds by absorbing the sample in methanol containing bromine and determining the dibromide formed (*354*). Such a technique has been applied successfully to the analysis of a mixture of ethylene and vinyl chloride (*355*).

In work on the effect of substituents on half-wave potentials, values are reported for a series of polychlorobenzenes (*253*), monohalogenated naphthalenes (*252*), and substituted iodobenzenes (*60*). The last investigation found that a linear relationship was obtained between the half-wave potentials and Hammett's sigma functions and that the fluorine in *p*-fluoroiodobenzene is reduced before the iodine.

The polarographic determination of γ-hexachlorocyclohexane is still receiving considerable attention. Studies have found that the sensitivity of the method is higher if the solvent contains alcohol or acetone rather than dioxane (*359*), that heptachlorocyclohexanes do not interfere in the analysis at a mercury pool electrode (*400*), and that with suitable modifications the analysis can be carried out without temperature control (*463*). Impurities, which have an effect on the analysis, can be either compensated for by comparing a saturated solution of the pure γ- isomer with one containing the technical sample (*447*), or removed by chromatography on silicic acid (*344*).

The analysis has been applied to cattle dips (*445*), kerosine solutions (*113*), commercial sprays (*111*), and mixtures with DDT (*461*, *462*). The method has also been used to check the values obtained for the γ- isomer by the dielectrometric method (*303*), to estimate the free energy change of the γ- isomer (*373*), to follow its formation in the chlorination of benzene (*117*), and to compare half-wave potentials with insecticidal activities (*465*).

The 6-electron reduction of the γ- isomer has been verified again using a microcoulometric reduction (*203*).

Reports are also given on studies of other types of halogen compounds. The polarographic behavior is reported for the reduction of trichloroethanol (*90*), and of α-halogenated acetaldehydes (*91*), butyraldehydes, heptaldehydes (*103*), cyclopentanone, and cyclohexanone (*315*). The shift of the half-wave potential for trichloroethanol in buffers with a pH greater than 11 pointed to an acidic nature for this alcohol. The kinetic currents obtained for the aldehydes are in agreement with their hydrated structure.

The method is suitable for determining chloral in the presence of dichloroacetaldehyde and chloroacetaldehyde (*88*).

In the α-bromoaliphatic acid series the effect of branching on the reduction has been studied (*93*) and the parameters have been calculated for the polar effects involved (*416*). The reduction of *meso*- and *dl*-α,α′-dibromosuccinic acids and their esters to fumaric acid and the ethyl ester, respectively, indicates that the polarographic elimination reaction is nonstereospecific (*95*).

The polarographic method is the basis for the coulometric determination of trichloroacetic acid (*283*), and has been used to follow the photochemical hydrolysis of chloroacetic acid by determining the chloride ion formed (*204*).

The reported reduction of chloroacetamide and dichloroacetamide at the same point is unusual, in view of the fact that trichloroacetamide gives three waves (*224*).

The determination of 3,3′,5′-triiodothyronine has been shown to be possible (*98*), and the work on the reduction of the carbon-halogen bond has been summarized (*87*).

NITRO COMPOUNDS

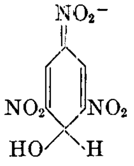
Aliphatic nitro compounds are found to give more stabilized waves in the presence of lanthanum salts (*288*).

In the aromatic series the mechanism of reduction has been verified for a large number of nitro compounds coulometrically, and has been studied more thoroughly for the second wave. The stability of phenylhydroxylamine in 0.1*N* hydrochloric acid precludes the possibility that the rearrangement of this compound to *p*-aminophenol is responsible for the partial kinetic current found for the second wave in acid medium. The pK of approximately 3.2 for phenylhydroxylamine (*20*) suggests that the following reaction may be involved in this phenomenon and may also explain the smaller second wave found in less acidic buffers.

$$C_6H_5NH_2OH \rightleftarrows C_6H_5NHOH + H^+$$

Oscillographic studies indicate that two consecutive processes are involved in the reduction, with the potential being determined by the reduction of nitrobenzene to nitrosobenzene (*409*).

The unique reduction found for *p*-dinitrobenzene in alkaline medium (*161*) has been studied on a large scale (*162*), and occurs also for 2,5-dinitrotoluene and 2.5-dinitroanisole but not for dinitrodurene and 2,5-dinitrophenol (*372*). Alkali also lowers the double wave for *m*-dinitrobenzene (*237*) and the triple wave for trinitrobenzene (*160*). In the last example hydroxylated *aci*-nitro compounds of the following type (X) are considered responsible for this behavior:



X

Studies have been reported on the reduction of the three nitrobenzaldehydes (*73*), *p*-nitroacetophenone and reduction products involving the nitro group (*125*), *m*-nitrobenzenesulfonic acid (*225*), *p*-nitroanthranilic acid (*208*), dipicrylamine (*312*), the three nitrophenylthiocyanates (*14*), and humic acids obtained by the oxidation of coal with nitric acid and air (*58*). Of these examples *p*-nitroacetophenone, *p*-nitrobenzaldehyde, and *o*-nitrobenzaldehyde give phenylhydroxylamines which can be reduced in all buffers to the corresponding anilines.

The method has been used to estimate 2,4-dinitro-6-cyclohexylphenol in a mixture with 2,6-dinitro-4-cyclohexylphenol (*274*, *277*, *466*), to study hydrogen bonding in these compounds (*228*) and other aromatic nitro compounds (*158*), to follow the hydrolysis of *p*-nitrophenyl acetate and *p*-nitroacylanilides (*159*), and to determine chloramphenicol in blood and urine (*214*), trinitrotoluene in air, its metabolite in urine (*348*) and blood (*114*, *346*), and nitroanilines which are used as sweetening agents (*281*).

Polarographic analysis combined with separations by countercurrent distribution and chromatography has been used in the analysis of mixtures of 2,4-dinitrochlorobenzene with *o*-nitrochlorobenzene and with *p*-nitrochlorobenzene (*201*), of the nitroanilines, of the nitrophenols (*206*) and of *m*-dinitrobenzene with nitrobenzene (*2*).

The reduction of aromatic nitro compounds is the basis for the indirect determination of toluene in blood and urine (*396*) and of *p*-cresol in urine (*18*). In both examples nitration converts these compounds to the corresponding dinitro derivatives. In other indirect applications *m*-nitrobenzoic acid is the basis for the determination of zirconium (*130*), and picric acid, flavianic acid, and sodium alizarin sulfonate are used for the determination of alkaloids (*495*).

Studies have also been carried out on the reduction of nitrophenols and nitrophenylarsonic acids at the streaming mercury electrode (*340*), and on the decomposition of nitroguanidine and nitrourea with pH (*295*).

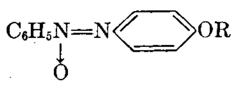
Half-wave potentials for the three nitrotoluenes, tolualdehydes, and iodotoluenes have been correlated with electron densities and activation energies for electrophilic substitution (*123*), and adsorption of the three nitrobenzoic acids has been studied using the electrocapillary curve of mercury (*173*).

NITROSO COMPOUNDS

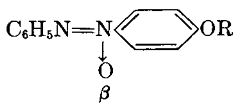
Polarographic reductions are reported for *p*-nitrosodiethylaniline (*209*), 1-nitroso-2-naphthylamine (*307*), 1-nitroso-2-naphthol (*460*), and neocupferron (*N*-nitroso-1-naphthylhydroxyl amine) (*48*).

AZOXY COMPOUNDS

Azoxybenzene has been found to undergo a two-step 6-electron reduction in acid medium to aniline. In other buffers the reduction proceeds to hydrazobenzene (*64*, *450*). With unsymmetrical azoxybenzenes such as *p*-hydroxy- and *p*-methoxy-azoxybenzenes the α- isomer is more easily reduced than the β- isomer (*65*). Work on these compounds and the corresponding azo compounds (*66*) has been summarized (*67*).



α

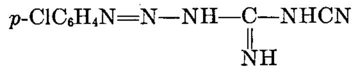


β

DIAZONIUM SALTS AND AMINOAZO COMPOUNDS

The effect of substituents on the reduction of diazonium salts has been studied (*216*) and the 4-electron reduction to phenylhydrazine has been verified on a large scale (*352*).

Work on *p*-chlorophenyldiazocyanoguanidine (XI) indicates that the two tautomeric forms reduce at different points (*335*).



$$p\text{-}ClC_6H_4N{=}N{-}NH{-}\underset{\underset{NH}{\|}}{C}{-}NHCN$$

XI

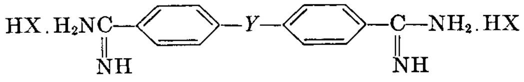
AMINES, AMINO ACIDS, AND DERIVATIVES

The determination of amines and amino acids polarographically continues to be carried out indirectly. Secondary amines like dimethylamine (*245*), diphenylamine, and *N*-ethylaniline (*390*) are determined through their *N*-nitroso derivatives. Methylamine (*245*) can be determined by converting it to the piperonal derivative. The decrease in the phthalaldehyde wave brought about by ammonia (*302*) and amino acids (*301*), and in the copper wave by propylenediamine (*166*), is used as the basis for evaluating these nitrogen compounds.

The reduction of dodecyltrimethylammonium chloride is reported (*386*), and that of tetraethylammonium iodide found to occur at more negative potentials than that of tetramethylammonium iodide (*205*).

Directions are given for the purification of tetramethylammonium chloride and iodide (*324*, *431*), and for the preparation of phenyltrimethylammonium hydroxide (*110*) and tetrabutylammonium iodide (*285*).

The reduction reported for a series of amidines (XII) (*238*), in which *Y* varied in structure, is probably due to the discharge of hydrogen ions.



XII

Catalytic waves are reported for the gonadotropins which have a protein structure (*321*), and anodic waves are observed for proteins in acid medium which can be used for their characterization (*196*).

PHOSPHORUS COMPOUNDS

Quaternary phosphonium salts, $R_4P^+I^-$, are reported to undergo a 2-electron reduction (*59*).

UNSATURATED HYDROCARBONS

In the aliphatic series the method has been used to detect cyclooctatetraene in the photopolymerization of acetylene (*239*), reports a reduction wave for a hydrocarbon which is either cyclodecapentaene or bicyclo-(6,2,0)-2,4,6,9-decatetraene (*293*), and fails to distinguish between vinylcyclooctatetraene and cyclooctatetraene (*126*).

Work in the aromatic hydrocarbon series using 96% dioxane (*155*) or anhydrous dimethylformamide (*448*) has indicated that the reduction of these compounds in 75% dioxane, reported previously, proceeded by the following steps:

$$R + e \leftrightarrows R^{-}$$

$$R^{-} + H_2O \rightarrow RH + OH^{-}$$

$$RH + e \rightarrow RH^{-}$$

$$RH^{-} + H_2O \rightarrow RH_2$$

In 96% dioxane the second reaction for anthracene becomes slow and the reduction proceeds in the following manner:

$$R + e \rightleftarrows R^{-}$$

$$R^{-} + e \rightarrow R^{=}$$

$$R^{-} + 2H_2O \rightarrow RH_2 + 2OH^{-}$$

A similar behavior was observed for stilbene in dimethylformamide and was verified by the isolation of a dimer of the anion free radical in a large-scale electrolysis.

The importance of the first step, which actually determines whether a compound will show a reduction wave or not, in determining the exact value of the half-wave potential, has been questioned recently (*24*).

The study of aromatic hydrocarbons has been extended to polyphenyls (*154*), diphenylpolyenes (*153*), and biphenylenepolyenes (*151*). The stepwise addition of electrons to dibiphenyleneethylene reported previously has been verified by potentiometric titration of this compound with sodium biphenyl (*152*).

Work on 78 polycyclic aromatic hydrocarbons in methyl Cellosolve found that plots of the half-wave potentials versus the wave number of their absorption spectra are linear for related hydrocarbons (*19*). The values reported for 1- and 2-cyclopentenyl and cyclohexenyl naphthalenes parallel their reactivity in the Diels-Alder reaction (*212*).

The method has been used to analyze indene and coumarone fractions (*330*), is suitable for the determination of anthracene in coal tar products (*128*) and for predicting tumor potencies of high-boiling petroleum fractions (*84*), and has been used to follow the alkaline condensation of indene with benzaldehyde (*331*).

The oxidation of the leuco base of Crystal Violet at the rotating platinum electrode is reported to proceed in a 1-electron step (*1*).

PEROXIDES AND HYDROPEROXIDES

The use of the polarographic method in studying peroxides and hydroperoxides has continued in several ways. Fundamental work on the reduction of hydroperoxides, peroxides, peracids, and α-hydroxyperoxides has been reported in aqueous medium (*43*) and in a benzene-methanol mixture (*22, 316*).

The method has been used to follow the decomposition of benzoyl peroxide in the presence of various substances (*424–426*), the action of catalase and ferrous ions on nine organic peroxides (*23*), the action of potassium ferrocyanide on cumene hydroperoxide (*28*), and to study the autoxidation of dioxane (*427*), methyl oleate (*313*), fats (*189*), and α-pinene (*21*).

In the oxidation of ethane, propane, pentane (*122*), 2-butene, and butane (*79*), the method has been useful in following the formation and in demonstrating the presence of peroxides (*336*) and aldehydes.

Studies have found that the peroxide from butyl methacrylate has a hydroperoxide structure (*30*) and that hydroperoxides can be determined in the presence of hydrogen peroxide (*44*).

Ascaridol, which gives a good wave in 70% ethyl alcohol (*275*), can be determined in oil of chenopodium (*276*).

The polarographic method for ascaridol has been compared with the iodometric method (*13*), and that for peroxides in fats has been compared with the Wheeler iodide and the stannous chloride methods (*338*). In the latter comparison the polarographic method gave more reliable values because it was more specific.

SULFUR COMPOUNDS

The polarographic reduction of disulfides which involved 2 electrons (*61*) has been compared with the behavior of trisulfides and tetrasulfides (*197*).

The reduction of cystine, which gives two waves, occurs according to the following equation:

$$R—S—S—R + 2e + 2H^{+} \rightleftarrows 2RSH$$

The prewave formed is explained by the reduction of the oxidation product between mercury and the adsorbed cystine (*190, 221*).

$$R—S—S—R + Hg \rightarrow Hg(SR)_2$$

$$(RS)_2Hg + 2e + 2H^{+} \rightarrow Hg + 2RSH$$

The current for cystine is lowered by the presence of amyl alcohol, dibutylamine, and sulfosalicylic acid (*273*).

Other reductions are reported for bis-(diethylthiocarbamyl)-disulfide (*493*), cystamine oxide (*255*), aryl thiocyanates (*14*), diaryl sulfones (*254*), and phenyl isothiocyanate (*469*).

Oxidation of organic sulfides at a platinum electrode is reported to give sulfoxides. The system is not reversible (*298*).

Compounds with sulfhydryl groups give anodic waves involving the formation of a mercury complex. Cysteine, which has probably been studied the most, gives an anodic wave at the convection electrode (*222*), two waves at the dropping mercury electrode, and only one at the vibrating platinum electrode. The second wave at the dropping mercury electrode is due to film formation (*132*).

2,3-Dimercaptopropanol (BAL) similarly gives an anodic wave involving 2 electrons and corresponding to the formation of a 1:1 complex with mercury (*494*). The quantitative estimation of this compound is best carried out at pH 4 (*49*) to 4.7 (*494*), is quicker than the potentiometric method but less accurate (*491*), and has been used in studies of its complexes with heavy metals (*490*).

The method has been used to study the reduction of cystine and dithioglycolic acid in the presence of iron (*223*), and to determine equilibrium constants for the reaction between oxidized glutathione and sulfite (*405*), and for the exchange reaction of cystine with glutathione and thioglycolic acid (*219*). The technique was involved in the determination of the structures of iron-cysteinate (*417*) and iron-thioglycolate (*251*) complexes, in the study of the reaction between cysteine, reduced glutathione and mercury, (*402*) and between thioglycolic acid and mercury (*404*), and is the basis for the amperometric titration of glutathione (*220*) and disulfides (*406*).

In natural products applications have been made to the determination of sulfhydryl compounds in fruits (*475*), plant materials (*341*), culture media (*392*), and etiolated seedlings (*127*), and to the demonstration of this group in pantetheine (*446*).

An indirect method for the sulfhydryl group in proteins is based on the combination with and the polarographic reduction of sodium *p*-mercurichlorobenzoate (*278*).

A number of sulfur-containing acid derivatives have been found to behave like the simple mercaptans and give anodic waves involving the formation of complexes of mercury. Such a behavior is reported for potassium ethyl xanthate, $KSCSOC_2H_5$ (*112, 357*), sodium diethyldithiocarbamate, $NaSCSN(C_2H_5)_2$ (*70*), sodium dialkyldithiophosphates, $NaSPS(OR)_2$ (*266*), thiourea (*85, 182*), and substituted thioureas (*182*). Compounds of this type are the basis for the determination of carbon disulfide (*492*) and carbon oxysulfide (*379*) after absorption in an ethanolic solution of diethylamine, and are formed from amino acids and carbon bisulfide (*471*). The determination of the last class is best carried out at pH 11 and can be used to determine the purity of amino acids (*470*).

The method for carbon oxysulfide has been used in a viscose plant (*347*), and that for carbon disulfide can be carried out oscillographically (*148*).

Sulfur in organic compounds can be determined on a micro scale by treating the compound with Raney nickel, separating the nickel sulfide, and determining the hydrogen sulfide content polarographically after dissolving the nickel sulfide in acid (*422*).

The Brdicka test has been used in varied investigations of proteins (*45, 119, 163, 164, 184, 185, 229, 282, 286, 291, 329, 345, 350, 367, 368, 398*) and diseases (*246, 268, 366, 384, 403, 474*).

The use of a zinc salt in the Brdička test is reported to make the test for cancer more specific (*139*).

The catalytic effect of cysteine in this test has been studied (*186*) and the maximum produced is found to be suppressed by alcohols, glycols, and polyoxyethylenes in amounts that are characteristic of each substance (*454, 455*).

The use of graphite, molybdenum, antimony, and bismuth reference electrodes has been investigated in studies involving the anodic waves for sulfur compounds (*181*).

ORGANOMETALLIC COMPOUNDS

Polarographic studies are reported on alkyl tin compounds (*421*), triethyltellurium chloride (*388*), triphenylselenium chloride (*387*), dicyclopentadienylnickel (*459*), biscyclopentadienyltitanium dibromide, biscyclopentadienylniobium bromide (*458*), bisindenylcobalt perchlorate (*319*), and the catalytic reduction product of vitamin B_{12} (*179*).

The method has been used to study the reaction of triphenylselenium chloride with bismuth triiodide (*385*) and to determine the composition of triphenylselenium iodobismuthite (*389*).

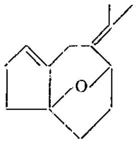
The method has been used indirectly in the determination of tetraethyllead (*7, 169*) and for lead in lead soaps (*394*).

OXYGEN- AND SULFUR-CONTAINING HETEROCYCLIC COMPOUNDS

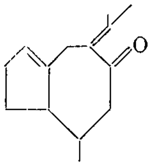
In the furan series fundamental investigations have been carried out on furfural (*71*), furacin(5-nitrofurfuraldehydesemicarbazone) (*364*), and phenyl furyl ketone (*72*). The method finds furacin is best determined at pH 2.9 to 3.2 (*289*) and has been applied to the determination of furacin in biological materials (*333*), and to the detection of furfural in browned orange juice (*300*). Comparison of the bactericidal action of nitrofuran derivatives and their half-wave potentials with nitrobenzene analogs indicates that the furan derivatives are better bactericidal agents and more easily reduced polarographically (*365*).

Reductions in the thiophene series are reported for 2,2′-dithienyl ketone (*72*) and thenoyltrifluoroacetone (*92*). In arriving at the mechanism of reduction for the latter, comparisons in behavior were made with that of methyl thienyl ketone, thenoylacetone, and acetylacetone.

Germacrol (XIII) is the only oxide in the terpene series which is reducible polarographically (*304*). This compound is easily converted to germacrone (XIV), which is found to the extent of 2.5% in zdravetzol.



XIII



XIV

The polarographic method has been applied to the determination of *O,O*-diethyl-*O*-(4-methylcoumarinyl-7) thiophosphate and its methyl analog (*233*) and of meconic acid in opium (*280*), and to a study of the effect of alkali on the opening of the coumarin ring (*107*).

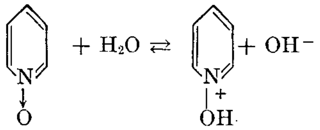
In the flavone series investigations are reported on the reduction of myricitrin, rutin, myricetin, quercetin, hesperidin (*413*), and the flavones in Chinese citron juice (*299*); the same factors operating in changing the infrared frequency of the carbonyl group are found to be responsible for the ease of reduction polarographically (*142*).

The copper complex formation of flavones is a basis for their amperometric titration (*76*).

NITROGEN-CONTAINING HETEROCYCLIC COMPOUNDS

Polarographic studies are reported for α-pyrrole aldehyde (*54*) and α-methylenedihydroindole (*97*). The reduction reported in the latter paper for *N*-vinylcarbazole may be due to peroxides.

In the pyridine series, pyridine gives a kinetic wave in unbuffered medium and a kinetic wave and a hydrogen discharge wave in acid (*242*). 2,2′-Bipyridine is reported to give a 2-electron reduction wave in alkaline medium (*391*), whereas propylpyridinium salts show a 1-electron reduction (*62*). Pyridine oxide exhibits a partial kinetic current and is reduced to pyridine. The results suggest that the following reaction may be responsible for the kinetic character of the wave (*108*).



Introduction of carboxyl groups into the pyridine ring modifies the polarographic behavior. Studies are reported on isonicotinic acid, picolinic acid (*202, 294, 444*), quinolinic acid, and cinchomeronic acid (*440*). The amides of picolinic and isonicotinic acids behave similarly and can be determined in the presence of the acids (*180*). The oxides of picolinic, nicotinic, and isonicotinic acids resemble pyridine oxide and are reduced to the corresponding acids (*109*).

Other pyridine derivatives studied are anabasine (*257*), nicotinamide methiodide (*244*), and diphosphopyridine nucleotide (*55*).

The method has been used to determine iron nicotinate in pharmaceutical preparations (*195*), diphosphopyridine nucleotide in normal and malignant tissue (*56, 57*), and isonicotinic hydrazide (*430*).

The technique is not successful with mixtures of pyridine derivatives, but the oscillographic method gives good results (*443*).

Coulometric analyses indicate that the reduction of quinoline, isoquinoline, and quinaldine involves 2 electrons at all pH values (*407*).

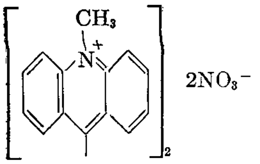
Polarographic data are also reported in this series for sinomenine (*282*), papaverine (*25*), Percain[2-butoxy-4-(2-diethylaminoethylcarbamoyl)quinoline hydrochloride] (*82*), Atophane(2-phenylquinoline-4-carboxylic acid) (*83*), cotarnine, hydrastinine, berberine (*68*), quinine, quininone, quinotoxin, quinotoxyl (*10*), quinoline-6- and quinoline-7-carboxylic acids and aldehydes (*325*), 8-quinolinol (oxine) (*473*), chelerythrine, and sanguinarine (*12*).

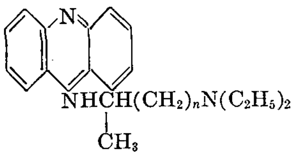
Determinations are reported using the reduction of nitroso derivatives as a basis for cephaeline (*133, 183*) and *N*-allylnormorphine (*442*). Diacetylmorphine can likewise be determined in this manner after hydrolysis and conversion to nitrosomorphine (*279*).

The determination of brucine and strychnine is possible using catalytic waves after separation of the compounds chromatographically (*200*).

Polarographic data obtained for quinine hydrochloride have been used to test an equation derived for catalytic currents (*265*).

In the acridine series the method has been applied to a study of the mechanism of oxidation of lucigenin (XV) (*236*), and of the hydrolysis of mepacrine (XVI) and three of its homologs ($n = 1$-4) (*136*).


XV

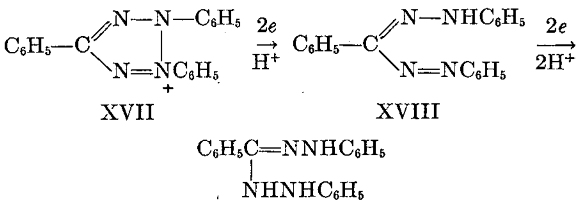

XVI

Polarographic studies of cyclic compounds containing more than one nitrogen are numerous. Results are described for uracils (*267*), adenine (*106*), barbiturates (*194, 486*), thiobarbiturates (*479*), 8-chlorotheophylline (*428*), oxides of benzophenazine and phenazine (*69*), phenazinones (*349*), and Janus Green B, a dye that is used for mitochondrial staining (*63*).

Work in this field has confirmed the conclusion made by acid titration, that zinc is combined with the imidazole group in insulin (*418*), has found that thiobarbituric acids have varying abilities to suppress oxygen and ascorbic acid maxima (*480*), and has been used to follow the structural changes produced in purines and pyrimidines by reducing agents (*135*).

The method has produced luminescence around the mercury drop in the reduction of oxygen in the presence of 5-aminophthalhydrazide (*438*), has demonstrated the presence of hydrogen bonding in nitrobenzotriazole (*287*), and has followed the decomposition of deoxyribonucleic acid (*262*) and the reaction of the latter with berberine (*213*). Determinations have been made for folic acid in whale liver (*308*) and for cyclotrimethylene trinitramine (RDX) (*187*). The latter determination was used to follow the alkaline decomposition of RDX.

In the reduction of triphenyltetrazolium chloride (XVII) a total of 4 electrons is involved and benzhydrazide (XIX) is reported to be formed.


XVII XVIII XIX

The intermediate, formazan (XVIII), gave an anodic wave which suggested that the first step was reversible (*177*). A similar study was carried out with various substituted triphenyltetrazolium salts and the results were compared with the corresponding phenylhydrazones (*339*).

Vitamin B_1, which gives an anodic wave at the dropping mercury electrode resulting from the formation of a mercury complex, forms no wave at the vibrating platinum electrode (*328*) but gives the Brdicka test (*351*). The method can be used quantitatively for vitamin B_1 in pharmaceutical products (*326*).

In order to obtain more data on the behavior of vitamin B_1, compounds related to this compound in structure have been studied with respect to oxidation, reduction, and the Brdicka test (*414*).

Reductions are reported for 2-acetamido-5-nitrothiazole (*171*) and 10 chlorophyllins (*464*).

The oscillographic method is rapid in assaying the purity of penicillin G (*145*).

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Instrumentation

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THIS review is confined to general principles and techniques of instrumentation which are of direct or indirect interest and importance for the analytical chemist. As before, this approach is intended to avoid duplication of topics which are treated by other reviewers of special methods of analysis, in which they have necessarily covered the instrumentation pertaining to the particular technique.

The topics are grouped under the general headings of transducers, electronics, servomechanisms, recording and controlling, computers and data assimilation, special techniques, and materials. A period of approximately 4 years is covered by these topics. The treatment is selective rather than exhaustive or complete, for the reason that many important instrumental developments have no foreseeable use for the analyst, although in their original intent or field of application they are equally useful and significant.

TRANSDUCERS

A transducer or primary element is any device which will convert the phenomenon under investigation into an appropriate signal, preferably electrical. There is a growing need for a comprehensive treatment or discussion of known transducer elements. A useful and important step in this direction has been made by the DuMont Co. (*51*) in listing analog transducers which are suitable, in one way or another, for the presentation of a phenomenon on a cathode-ray oscillograph. The majority of these transducers can be used in other ways and are not exclusively limited to cathode-ray tube presentation. Over 500 different types of analog transducers are listed in this compilation. For each transducer, the following information is given as available from the manufacturer: function, principle of operation, accessories required, transfer characteristics, power required, amplitude range, sensitivity, output characteristics, band width, resonant frequency, resolution or precision, linearity, weight, range, sturdiness, temperature limitations, mounting, size, remarks, and model designation. A separate section tabulates Geiger-Müller tubes and another contains pertinent reference material quoting authors and dates of publication.

A very useful application of the resistance wire strain gage has been made by Davidson (*27*) in constructing a simple cantilever beam balance. Although he used it for measuring the contraction of muscle, the latter was an indirect means of an analytical determination. A modification of Davidson's instrument was used by Müller (*74*) for automatically recording the imbibition of liquids in filter paper strips. As a further step, a simple electrical modification (*76*) permitted the automatic recording of the square of the weight as a function of time. Another application of the strain-gage principle uses the Statham pressure transducer to replace the manometer in the Warburg apparatus (*4*).

The strain-gage principle is susceptible to high precision measurements far beyond the usual operating practice. Hamon (*42*) has described an elegant circuit for detecting changes in resistance of these elements to 1 p.p.m. He uses the Waidner-Wolff adjustable resistance elements in the measuring arm of the bridge circuit instead of the more usual slide-wire. A very convenient provision is made for an initial adjustment of a 2% difference between active and dummy gages without altering the bridge adjustment for a given strain.

The great sensitivity and versatility of thermistors as temperature-sensitive elements are leading to many useful analytical applications. Most of the early objections to their use have disappeared as investigators have learned how to use them properly. A most useful application has been described by Zemany (*115*), in which a thermistor is used as a pressure gage to determine molecular weights by means of an effusiometer. It permits the molecular weight determination of a few hundredths of 1 cc. of gas at N.T.P. with an average error of 2%. In a very simple glass assembly, the gas is introduced at reduced pressure and allowed to escape through a pinhole aperture into an evacuated region. A straightforward application of Knudsen's equation for molecular flow is shown to lead to the conclusion that, if the logarithm of the pressure of the effusing gas is plotted against time, a straight line is obtained, the slope of which varies inversely as the square root of the molecular weight. The thermistor gives a continuous indication of the pressure decrease.

Smith (*100*) has described the performance of thermistors used as hygrometers. Stolten and Müller (*80*) have developed a differential thermistor instrument for micromolecular weight determinations in solution. In this technique the thermistors measure the heat of condensation of saturated solvent vapor on the solution. The equilibrium temperature difference is exactly proportional to the mole fraction of solute. The preliminary studies of Stolten, which led to the development of this instrument, served to establish the high degree of reliability of the thermistor when it is operated under the proper conditions. A crucial point in this connection is to keep the thermistor bridge circuit excited constantly. Failure to do this may require hours or days to reattain equilibrium and precise and reproducible response to temperature changes. An important point to consider is the self-heating effect in the thermistor. Unless this is very small, disturbing effects can arise. This might be considered as an inherent defect in the thermistor as compared with a thermocouple. Actually, several useful applications are made of this dissipation effect, notably in anemometers or flow-measuring systems. The chemist or physicist may be inclined to view the thermistor as essentially a new thermometric element. Actually, the majority of its applications are in the form of temperature-compensating elements, and many ingenious schemes have been devised to render instruments and measuring systems immune to wide temperature fluctuations. There was a time when chemists used to hang everything in a thermostat. As far as instruments or measuring elements are concerned, this is no longer necessary. An extensive range of thermistor devices for measurement and control is sold by the E. H. Sargent Co., Chicago, Ill.

The copper resistance thermometer has been re-examined and designed in improved form by Dauphinee and Preston-Thomas (*26*). They show that fine commercial copper wire attached with Formel varnish to a copper base has sufficiently stable and reproducible resistance temperature characteristics to be used as a resistance thermometer in the temperature range 20° to 320° K. They have given the resistance ratio and the temperature coefficient of resistance for such thermometers in the form of a table for temperatures ranging from 4.2° to 320° K. They find that, whenever a copper resistance thermometer agrees with this table within 0.1° K. at the nitrogen point, the table gives the temperature coefficient of resistance within 0.1% at all higher temperatures.

New photoelectric devices are appearing; they are among our most useful and versatile transducers. Moreover, improvement by a remaining factor of less than 10^3-fold would be of tremendous significance in the widespread utilization of solar energy. A large-area germanium photocell is described by Pantchechnikoff (*84*), which consists of a germanium crystal soldered onto a stud which is held concentric with a short metal sleeve by an insulator. A thin, semitransparent metal film is evaporated on the germanium surface to form a rectifying and photosensitive contact. With a tungsten light source at 2870° K. a typical response is 10 ma. per lumen. The spectral response rises sharply at 1.9 microns, reaches a peak at 0.7 micron, and continues to decline throughout the visible. Operation has been achieved through a 125-ohm load at a frequency of several megacycles per second. From a tungsten source, the photocurrent is proportional to illumination up to at least 500 foot-candles.

The rapid advances in solid-state physics, both in theory and practice, have afforded new photoelectric devices. Thus, Lehovec (*64*) proposes two new components based upon the detection of injected carriers by their absorption and by emission due to their recombination with majority carriers. One of these is the photomodulator, which permits modulation of a light beam by the change in absorption due to injected carriers. Another is the "graded seal junction" which permits the light to be transmitted to and from a P-N junction without substantial absorption in the bulk semiconductor. Lehovec has suggested that the photomodulator combined with a phototransistor might be useful as an amplifier.

Of more immediate interest is the P-N junction photocell described by Shive (*95*). It has a sensitivity of 30 ma. per lumen for light of 2400° K. color temperature. This is practically unit quantum efficiency in the spectral range from the visible to the long wave cutoff at 1.8 microns. This cell exhibits a dark current of a few microamperes at room temperature with a positive temperature coefficient of 10% per degree centigrade. Both dark and photocurrents exhibit saturation in the range of 1 to 90 volts applied. Frequency response is flat into the 100-kc. region. The photocell element is encapsulated in a 1/4 × 3/16 × 3/8 inch plastic housing.

The measurement of small pressure differences and at high speed is described by the physiologist Pappenheimer (*85*). He has revived a 60-year-old idea and fashioned it into an elegant device. It is a differential conductance manometer which makes use of a diaphragm separating two electrolytic conductivity cells. Each conductivity cell consists of two chambers filled with electrolyte and communicating with each other by means of a narrow gap of the order of 20 microns. This gap is widened in one cell and correspondingly narrowed in the other cell as the result of the movement of the diaphragm in response to pressure differences. Inasmuch as two thirds or more of the resistance resides in the gaps, the system is extraordinarily sensitive to diaphragm motion. In a typical case an output signal of 1 mv. is produced by a diaphragm displacement of 0.07 micron for a bridge excitation of 1 volt. Correct design involves a consideration of hydraulic resistance to flow, the use of a trace of detergent in the electrolyte, and the removal of air bubbles. The transient response can be made very fast. A single example of the many uses of this transducer is selected here. When used with a differential osmometer of the Hipp type for determining the molecular weight of a dextran fraction (molecular weight, ca. 25,200) equilibrium was 95% complete in 45 seconds.

The glass electrode has been an important transducer for the chemist for many years. The transient response of the glass electrode to pH step variations has been studied oscillographically by Disteche and Dubuisson (*31*). It turns out that the response is independent of thickness of the glass (5 to 10 microns). Some evidence is adduced to show that the response of the electrode to pH steps is analogous to the response of a low pass *RC* filter to a corresponding voltage step, where *R* and *C* are, respectively, the resistance and capacitance of the glass membrane. In any case, the response seems to be so fast that the limitations are of a mechanical nature—i.e., to produce the step change in pH fast enough.

In the region of low pressures an extension of the early principle of A. H. Pfund (*88*), wherein a small Pirani gage was sealed into the capillary of a McLeod gage, has been made by Bradley (*12*) who has used a thermistor as the pressure sensing element. This modification of a McLeod gage is useful over the range of 1 to 10^{-7}mm. of mercury pressure. An auxiliary thermistor is mounted near the measuring thermistor for temperature compensation. A simple bridge circuit is used for balancing. The necessary compensating resistance varies linearly with the square root of the gas pressure. As it is primarily a Pirani gage, the readings are a function of gas composition as well. The author emphasizes the ease with which the system can be calibrated in absolute terms. It would seem that the simple square law computer of Müller (*76*) could be incorporated in the bridge circuit to make readings proportional to pressure instead of the square root of the pressure.

An unusual transducer using a gas discharge tube has been developed by the Decker Aviation Corp. (*49*) based upon some original investigations by Kurt S. Lion. In one form of the device a long discharge tube contains sealed-in electrodes at either end. A movable external ring electrode situated near the middle of the tube is connected to a radio-frequency exciting source and through two capacitors to the end electrodes. The glow discharge in the tube, produced by the radio frequency source, results in no potential difference between the end electrodes if the central ring electrode is symmetrically located between them, but a direct current potential will appear across the end electrodes if the central ring electrode is displaced. The output signal as a function of ring electrode displacement is of the order of 2 volts per microinch. The output versus displacement is linear and has directional discrimination—i.e., the potential reverses sign around the null position. In another version of the device, external excitation of the glow discharge is achieved and the output can be cancelled by trimming capacitors. Any small change of capacitance acting in parallel with either capacitor will alter the direct current output component. This then is another transducer sensitive to small displacements or equivalent changes in capacitance.

Hollmann (*47*) has written a most provocative paper on the subject of polaresistivity and polaristors, a phenomenon and devices based upon the properties of systems consisting of semiconductive particles suspended in an insulating fluid. Fibration can be induced by applied electric fields and the conductivity becomes nonlinear as soon as the fibration at a certain threshold field reaches a point at which the semiconductive fibers bridge from one electrode to the other. Solid systems of this sort have been prepared with various powders dispersed in thermo- or cold-setting plastics. These have been termed "polaristors." In one case, the use of titanium oxide of reduced oxygen content dispersed in Castolite revealed a 16-fold nonlinearity, which is far higher than that of Thyrite. The polaresistive systems are not only nonlinear, but are at the same time sensitive to temperature and mechanical strain or stress, so that new sensing elements and electromechanical transducers may be developed. The versatility of polaristors can be judged from the fact that one is at liberty to intermix a great variety of powders with various properties such as semiconductive ferromagnetic particles, photoelectric or photoconductive powders, and the like.

Related work on the production and properties of isotropic artificial dielectrics is described by Corkum (*24*). In these systems, small and very uniform conducting spheres are embedded in an insulator such as Styrafoam in precise geometric spacing. The structural dielectric so produced has unusual and interesting properties. The behavior of these materials is most conveniently studied in the microwave region. In the past, structural dielectrics have been made which exhibit fantastically high values of effective dielectric constant. They have found extensive use in the preferential reflection of radar signals.

The movable anode tube such as the RCA 5734 has found many uses as an electromechanical transducer. It finds widespread use as a phonograph pickup element. An interesting application is in the form of a sensitive pressure-measuring device for use in physiology. The instrument, described by Pettersson and Clemedson (*87*), provides an output signal of about 1 volt for a membrane displacement of 0.002 mm.

ELECTRONICS

As we all know, electronics is a vast subject and a specialty of its own. In selecting a few outstanding developments one may serve the present problem by indicating trends and the extent to which some of the newer developments are serving the analyst and may be expected to change some of his practices in the future. To a degree, there exists a practice of formal electronic circuitry in which an almost unlimited number of special-purpose circuits can be devised, all based upon standard and fundamental circuits. Applications to analytical chemistry could continue for many years on the basis of these alone. Aside from a few relatively new electronic devices, this discussion is confined to special circuits which have more or less immediate use for the analyst.

A new hot-cathode, helium-filled diode known as the Plasmatron has been developed by RCA. As described by Johnson (*55*) this tube is capable of controlling large currents continuously at low voltages. A small control current provided by an ordinary vacuum tube such as the 6J5 provides an auxiliary discharge in the Plasmatron, which provides ionization to neutralize space charge. The steady-state density of charged particles in the plasma depends upon equilibrium between generation and loss rates and is very nearly proportional to the magnitude of the auxiliary or controlling current. The presence of a plasma, of controllable density, between the main electrodes of the Plasmatron permits the passage of large currents between the main cathode and anode, even at anode potentials of a few volts. In a typical case, a control current of 20 ma. yields an anode current of 950 ma. at an applied potential of only 6 volts. The average slope of this curve corresponds to a current amplification of about 90 to 1. Experimental forms of the tube have been used in motor control, pulsing circuits, and other electronic applications.

Transistor theory and practice present a formidable problem to the analyst, although he is already beginning to make useful applications. A useful approach to the basic principles of transistors is afforded by the paper of Coblenz and Owens (*22*). Also, a very concise tabulation of transistor equations has been given by Stansel (*102*). Exact equations as well as useful approximations are given for a number of parameters. Although this tabulation presupposes that one is reasonably familiar with transistors, it does supply design information in very convenient form.

Further reference is made to a useful paper of Wallace (*33, 108*).

An important application of transistors is in switching operations. Kruper (*60*) has described this technique and its significance is apparent to anyone who uses a modern electronic recording potentiometer. In most low-level, direct current amplifiers it is customary to use an electromechanical chopper to switch the direct current input signal in alternate directions through the primary of a transformer. The output of the latter can then be subject to alternating current amplification and operate the balancing servo-system. This chopping operation can be done electronically by transistors with a number of resultant advantages, if an alternating current signal (square wave) is applied between the transistor base and collector. Although there are many advantages, which cannot be described here, they all exemplify the usual superiority of purely electrical over electromechanical devices.

An elegant example of modern instrument amplifier design as embodied in several Leeds & Northrup indicators and recorders is described by Williams, Tarpley, and Clark (*113*). There are now several well-known instruments of this class where no provision is made for zero drift, for the reason that there is none! This comes about through meticulous attention to the design of the first stage in the amplifier, plus a very high degree of inverse feedback.

Logarithmic amplifiers are useful especially where a phenomenon must be followed over several orders of magnitude. It has been known for a long time that a logarithmic relationship exists between the current through a diode and its anode voltage when the latter is negative. This phenomenon has been reinvestigated in the region of very weak currents (*15*). By using a multigrid electrometer tube in diode connection and at subnominal rating, it is found that the logarithmic current-voltage curve extends over 5 decades. With some tubes, currents as small as 10^{-14} ampere follow this relationship. The circuit is particularly useful with phototubes or ionization chambers.

The use of an electrometer tube in conjunction with either a pentode or triode in cathode-follower arrangement is described by Brewer (*14*). For input currents of the order of 10^{-14}A. the output can be read on an ordinary voltmeter or recorder. The voltage gain is 0.9 and the input capacitance is a small fraction of 1 micromicrofarad.

In the classical approach to electrical measurements, one always referred to a standard cell as a reference source of e.m.f. We are by no means free of this device, but many modern electrical and electronic systems require a wide range of reference voltages the reliability of which approximate the standard cell. One can get a close approximation to this by elaborate electronic circuitry. A résumé of electronic practice of this nature up to 1948 is available in a monograph (*73*). A recent development by the General Precision Laboratory, Inc., is an all-electronic "secondary standard cell," useful as an adjustable voltage reference source. It has output ranges of 0.1, 1.0, and 10 volts, direct current full scale, with an output impedance of 0 to 1000 ohms, depending on dial setting with an accuracy of 0.1% full scale. Each division of the dial corresponds to 0.001 of full range.

The use of voltage regulator tubes, in which the potential across the gas-discharge tube is independent of current through the tube over a wide range, is a widespread practice in electronics. A useful and practical means of computing circuits for their rapid utilization has been published by Miles (*72*). He has been kind enough to use both the old (V.R.) and the new (confusing) type designations for these tubes.

Timing operations have been handled electronically for many years and, in the microsecond range or less, electronic schemes have few competitors. Even intervals of a few seconds can be treated conveniently and Armstrong (*3*) has described an arrangement of two gas triodes with RC coupling and a relay for this purpose. The action is similar to a multivibrator, in that the relay is actuated by the firing of one tube and switches itself to the action of the second tube which, upon firing, releases the relay and starts operations over again. Only one set of relay contacts is required for the cycling operation, so that, if a double-pole, double-throw relay is used, the extra pair of contacts can operate external control or timing operations. Like the ordinary multivibrator, this circuit can be arranged in a stable, monostable, or bistable form. In the first, the circuit will be free-running, at a rate dependent upon circuit constants. In the second, or monostable form, the relay will be either open or closed and remain in this state indefinitely until an actuating pulse is received. It then switches abruptly and after a definite time will switch back again to the original stable state. In the third mode, the bistable form, the relay will remain open or closed indefinitely. Actuating pulses are fed to a common trigger input connection and the effect of an incoming pulse will be to switch on that tube which happens to be in the nonconducting state.

A similar technique suggested somewhat earlier by Merlis and Degelman (*71*) was used as an artificial respirator for animal experimentation, although it has obvious chemical applications. The instrument is an automatic device for alternately admitting air or other gases to a system and then connecting the system to exhaust at rates varying between 5 and 40 cycles per minute. A double-ended piston in the pump is driven by solenoids. The latter are excited alternately by current from a tube which in turn is controlled by pulses from a multivibrator. A relay is also used in the intermediate tube circuit, but the authors suggest a modification wherein 6L6 power tubes can operate the solenoids directly.

It may not be out of place to point out that multivibrators and related electronic switching and timing devices have so many diversified functions and applications that the art is almost a special branch of electronics. Many operations, which would be cumbersome and expensive if performed mechanically, can be accomplished with ease and precision by means of trigger circuits.

Although galvanometers are rapidly disappearing from the research laboratory as they are replaced by more rugged electronic indicators, one still finds means suggested for improving their sensitivity. Thus, Jervis (*53*) has described still another phototube amplifier attachment in which an auxiliary light source projects a second beam of light on the galvanometer mirror and a rectangular image 11 x 5 mm. is received by a split cathode phototube such as the RCA 120. The two photocurrents feed a twin-cathode follower stage, resulting in a 1-ma. change in a 5000-ohm load per mm. deflection of the galvanometer. The arrangement does not interfere with the usual lamp and scale presentation. The amplifier output can operate a 0- to 1-ma. pen recorder. Readers will recall that the General Electric Co.'s Autopot is a commercial version of the galvanometer-phototube combination. Also, the Beckman Photopen recorder uses a basic Brown recorder chassis in which a pair of phototubes "chase" the galvanometer beam and drag a pen across the chart in exact proportion to the deflection.

The author has had occasion to use a variation of this principle in which the time at which a galvanometer had drifted to stated positions was automatically printed. In this case, a grid pattern from a Ronchi plate was projected on the galvanometer mirror and the image received on a slit in front of a phototube. The phototube was connected to a Schmitt trigger circuit and for the traverse of each light interval the output pulse actuated a printer, recording time.

Photomultipliers have found widespread use in many analytical instruments and it might be assumed that they represent the latest word in what can be accomplished in the measurement of very low light intensities and at high signal to noise ratio. It is, therefore, astonishing to note what can be done with the ordinary gas-filled tube by means of some tricky circuitry. Johnson (*56*) has developed what he calls a "snowballing" technique wherein a gain of 10^8 can be attained in a gas phototube with good stability and reproducibility. In his arrangement, regulated high voltage is applied to the gas phototube through the contacts of a Weston Electric mercury relay Type 276E, the coil of which is driven by 60-cycle alternating current. Under the high potential, the photocurrent increases in avalanche fashion as a consequence of primary photoelectrons, gas multiplication, secondary emission, and photons developed in the gas excitation. When the combined effects have "snowballed" to a value of about 1 μa., the high voltage is reduced to something of the order of 10 volts and the discharge ceases. This is accomplished automatically by passing the discharge current through a 600K resistor, the drop across which triggers a 2D21 thyratron and reduces the potential across the phototube. After some 8000 microseconds, the process repeats itself. The build-up time is the quantity which is measured, either oscillographically, or by circuitry which can convert build-up time into an output current or voltage. Build-up time and primary light intensity are related logarithmically in an expression which can be derived and is in agreement with the observations. It has been checked between the levels of 10^{-2} and 10^{-14} lumen. As might be expected, the data deviated from the predicted relationship at very low levels, owing to the small but definite thermionic emission at room temperature. If the phototube is refrigerated, the linearity extends to lower levels of illumination.

Photoelectric photometers with logarithmic response have been developed over a period of 20 years. Now one has appeared covering a range of 6 cycles. It provides continuous measurements from 10^{-3} to 10^3 microlamberts. As described by Plymale (*90*), a two-tube circuit in feedback arrangement automatically reduces the total dynode voltage of a photomultiplier, when the latter is exposed to light. Ordinary compensation over a range of somewhat less than 1000 to 1 was readily attained, but to reach a range of 1,000,000 special compensation had to be developed in order to correct the nonlinearity at low dynode voltages. The output of the system can be recorded and the wide range of response makes it ideal for such problems as measuring the decay rate of phosphors and related problems.

A novel use of optical feedback has been made by Seliger (*94*). In his scheme, the output of a photomultiplier is fed into an amplifier. A glow lamp connected in the plate circuit of one of the stages of the amplifier normally supplies a small but definite fraction of its light output to the photomultiplier along with the light which the photomultiplier is measuring. The feedback is degenerative—i.e., the intensity of the glow lamp diminishes as the output of the photomultiplier increases. Under these conditions, the glow lamp feeds back to the photomultiplier an amount of light necessary to cancel the increase or decrease from the primary source. The response in this case is linear in light intensity rather than logarithmic. In some applications, a logarithmic response is desirable; in others it may be objectionable. To avoid the zero balance problem common to direct current amplifiers, the author uses intermittent illumination as provided by a chopper. The optical feedback stabilization scheme involves no appreciable decrease in sensitivity.

Electronic proximity switches are most useful in the laboratory. In general, they consist of a high frequency oscillator which can be thrown out of oscillation by extremely small changes in distributed capacity to ground. An auxiliary tube closes a relay when the main oscillator tube stops oscillating. The Thermocap relay, manufactured by the Niagara Electronics Co., Andover, N. Y., has found widespread use in controlling temperature, pressure, linear displacements, and many other phenomena. The same principle is used in several moving vane pyrometers. An effect of humidity on such devices has been studied by Long (*66*), who shows that the effect is due primarily to adsorption on readily oxidized aluminum capacitor plates. This is the primary source of drift; the effect of moisture on the insulating studs, or of the known, but small, change of permittivity of the air, are not sufficient to explain the drift with humidity.

An electronic circuit for thermistor control of an 8-gallon water thermostat has been described by Noltingk and Snelling (*82*). The temperature-sensing thermistor of low heat capacity is connected in an alternating current bridge circuit, the output of which, after amplification, is fed into a phase-sensitive detector containing a grid-controlled rectifier which heats the bath directly. Change in amplifier gain does not affect the control point, only the sensitivity of control.

Phenomena occurring in the region of several thousand megacycles, the so-called microwaves, will become of increasing importance for the analyst just as all other regions of the electromagnetic spectrum have proved their particular advantages. Progress in this quasi-optical range of frequencies is great. An important series of developments is concerned with microstrip techniques which can, in certain cases, replace wave guides or coaxial structures. For the transmission of microwaves, the microstrip utilizes a single wire or strip supported above a ground plate. The extent of the field spread about the conductor is small and thus points to an economically compact configuration. Also, printed circuit techniques are applicable to this method and to the construction of inexpensive, noncritical microwave components. The new technique is discussed by Grieg and Englemann (*39*).

A simplified theory of microstrip transmission systems is presented by Assadourian and Rimai (*5*) and the design of specific microstrip components by Kostriza (*59*), who discusses three types of lines. The first is a cylindrical conductor suspended above a ground plane (wire above ground). The second is a narrow ribbon conductor separated from the ground plane by a dielectric material (strip line). The third is the same as the second, but with the strip immersed in a dielectric (dielectric sandwich line). All of the commoner microwave components such as transitions, standing wave detectors, attenuators, crystal mounts, directional couplers, and power dividers have been duplicated in the microstrip version. It is not to be inferred that the microstrip version will supersede wave guides or coaxial lines in all applications, especially at high power. The approach is most significant in the measurements field and does permit the extensive use of printed circuitry when the latter is used in the ground-plane conductor.

Precision frequency standards are of importance in many of our instruments. As in all phenomena, there are awkward or inaccessible regions for which no convenient reference elements exist. The piezoelectric quartz crystal cannot be ground conveniently to resonate at frequencies much below 50 kc. (It is true that frequency division can be effected by multivibrators with no loss in precision, but this is costly.) Similarly the precise, electrically driven tuning fork has a practical upper limit of about 5 kc. An elegant means of bridging this 45-kc. gap has been described by Dickson and Murdew (*30*) and depends upon the vibration of a taut wire. If an alternating current of appropriate frequency is passed through a taut wire while it is subjected to a strong transverse magnetic field, it will vibrate as a consequence of the reaction between the current and the magnetic field. As it vibrates, a counter e.m.f. develops across its length, which rises to a maximum as the frequency of the driving force reaches the resonant frequency of the wire. This change in e.m.f. causes the impedance presented to current sources to exhibit resonant characteristics. A systematic mathematical analysis of the equations of motion has been made by the authors; designs based upon these have been made and numerous applications are described. These elements can be used as high Q filter bridges and as discriminators whenever small frequency changes are to be measured. This is a fine example of instrumentation for its own sake. Perhaps the authors had no immediate problem to solve, which might account for its excellence and originality.

The advent of very small motors which can be run from the plate current of small vacuum tubes is a useful adjunct to many instruments. One of the best of these is a British variety, the low inertia motor supplied by Electro Methods, Ltd. A typical application of these has been made by Ward (*109*), who designed a portable integrating circuit for low radioactivity counts. These tiny motors will start rotating at a current of 100 μa. Ward describes two circuits. In the first, anode pulses from a halogen-filled Geiger counter operate a normal biased multivibrator pair with the integrating motor in the anode circuit of the second tube. For this arrangement, the shaft rotation is not a linear function of the true counting rate, because, at low rates, the motor actually stops between pulses. In the alternative circuit, pulses are fed to a capacitor, shunting the motor, through a germanium diode. Linear integration now results, but there is difficulty at very high rates in this method.

A wider study of integration by low inertion motors is well worth the effort. Prevailing methods of integration leave much to be desired; most of them are costly or very involved.

Ionic pumping is receiving considerable attention as a means of improving high vacua. It has several interesting instrumental aspects but also involves numerous curious and baffling fundamental effects. If residual gases are ionized and then accelerated by combined electric and magnetic fields, they can be driven into suitable absorbing surfaces with a consequent improvement in vacuum. In most arrangements, some sputtering of the electrode materials occurs and this assists in the clean-up process. It is not entirely clear, in any one technique, what the relative efficiencies of ionic pumping, mechanical entrapment, and chemical "gettering" action amount to; all of them seem to contribute to the reduction in pressure. Gurewitsch and Westendorp (*40*) give a brief description of the construction and use of such devices. Although the interpretation of the ionic pumping mechanism may be obscure, some startling results have been obtained. Thus Koch (*58*) separated and stored the isotopes of neon by using a mass spectrometer to drive the neon isotopes of different mass into separate metallic disks. Recently, Dieke at Johns Hopkins has taken advantage of the preferential diffusion of gases toward the cathode in a high voltage discharge. In rare gases, impurities tend to migrate faster toward the cathode. This cataphoresis, which Dieke prefers to call it, can lead to the purification of a rare gas to the extent of several parts in 10,000,000. Not only does he have spectroscopic and mass spectrometric criteria of purity in such cases, but, as has been known for a long time, the ionization potential of a rare gas is a most precise criterion of purity. Other studies in ionic pumping have been made by Schwartz (*93*) and Alpert (*1*).

Once more the advantage of series connection of phototubes in precise photometry is emphasized. This advantage was recognized long ago when Radio Corp. of America developed the 917 and 919 tubes in which the anode and cathode connections, respectively, were brought out of the glass envelope at the top. This improved insulation and permitted their direct connection by a very short lead. Glasser (*38*) has performed a useful service, nevertheless, by emphasizing the high photometric precision which can be attained with this technique.

SERVOMECHANISMS

The general principle of the closed-loop servomechanism is already embodied in many of our analytical instruments. Although it is not so defined by servomechanism experts, one may say that the principle involves nothing more than the classical method of the null or balancing method, in which the effect to be measured is balanced or opposed by an equal and opposite effect. The servomechanism does this automatically and continuously and without the intervention of an operator. The significance of this approach to measurement does not lie in any specific achievement, but emphasizes the fact that there is a unified and perfectly general method of attacking all classes of measurement. The subject is now so well understood, both theoretically and practically, that the servodesigner can solve almost any problem which is properly defined. The equipment which can control the level of anesthesia in an experimental animal on the basis of the alpha-wave output of an encephalograph is identical in a large portion of its components with the mechanism which controls the flight of an airliner, the navigational instruments of which are "locked" on a selected star. There is a vast gulf between electroencephalography and automatic celestial navigation, but aside from the specific nature of the transducers necessary in each case, and the different effect of other variables, the two mechanisms are remarkably similar.

The dozen or so examples which have been selected from hundreds of servo applications indicate a few achievements which are of specific interest for the analyst. The first group concerns weighing and the means for following continuously and automatically changes in weight. More than 30 years ago Oden, and Svedberg and Rinde in Sweden, constructed recording balances for following the intimate details of sedimentation. In 1935 Müller and Garman studied an all-electronic approach to automatic weighing. A complete instrument for this purpose was described later (*78*). Perhaps nothing has emphasized the importance and utility of continuous and automatic weighing as much as the advent of Clement Duval's monograph on Inorganic thermogravimetric analysis, published in 1953 (*32*). His techniques are being adopted throughout the world and several companies are developing recording balances as well as complete thermogravimetric assemblies. Industrial weighing devices have developed at a greater rate and they involve many ingenious schemes which might be translated into analytical requirements.

One of these is concerned primarily with a rate of flow problem. A change of weight criterion was chosen in preference to volume flow, and for this purpose a synchronous system responsive to scale unbalance was used to control a throttle valve determining the rate of flow. Pedersen (*86*) uses a linear variable differential transformer to detect unbalance of the scale. The amplified unbalance signal controls a motor which drives a weight along the balance beam, and a motorized valve, controlled by the detecting system, allows liquid to flow from the container. It is necessary to generate the first derivative of the error signal in order to obtain an error proportional to rate of flow. The maximum capacity of this instrument is 100 pounds with a sensitivity of 1 part in 100,000. Flow rates as low as fractions of a gram per minute up to several hundred grams per minute were achieved.

Fairbanks, Morse and Co., long noted for precise weighing equipment in the industrial field, has developed a 10,000-pound and 5000-pound scale which records ingredient weights on a printed tape (*21*).

At the other extreme of the weighing range, servo principles have been applied to the modernization of the gas-density balance. Most gas-density balances are null-point instruments for comparing gases at constant density. A new design by Simons, Scheirer, and Ritter (*96*) uses an electromagnetic device to apply a restoring torque to the beam. A capacitance null-point indicator shifts the frequency of an oscillator as the balance beam deflects. The output of this oscillator beats against a fixed frequency oscillator and the rectified beat frequency is a measure of deviation from the true null point. Electronic circuits permit this null-point detector to operate the restoring electromagnet automatically. These components have been incorporated in a practical gas-density balance. A change in weight of less than 0.1 γ can be detected. This corresponds, in terms of oxygen pressure, at room temperature to 0.02 mm. of mercury. In this self-balancing feedback system, a portion of the feedback current can be fed to a recorder.

An excellent paper by Mauer (*70*) describes the design of a recording analytical balance for following rapid changes in weight. It is being offered commercially by the Niagara Electron Laboratories, Andover, N. Y. This instrument utilizes a photoelectric criterion of the condition of balance and a feedback signal passes current through a solenoid which restores balance continuously by acting upon a permanent magnet suspended from one arm of the balance. Any desired fraction of the feedback current can be fed as an *RI* drop to a recorder. Damping is achieved electronically by a first derivative signal and this enables the balance to follow rapid changes in weight without oscillation. Mauer and his associates at the Bureau of Standards have made numerous applications of this instrument for combined thermogravimetric and differential thermal analysis studies. Apparently he shares this reviewer's concern about the wider use of such instruments. In a personal communication he states, "I have had about 300 inquiries, presumably from those who are considering building their own instrument. . . . An analysis of the types of laboratories is interesting. It seems that nearly everyone has something to weigh, but I suspect that some of the would-be users are only looking for a way of speeding up the process in conventional applications."

Peterson at the Mellon Institute has also designed a recording analytical balance of high precision. It employs a linear variable differential transformer as the sensing element. Numerous commercial instruments are now appearing in this country and in Europe, most of them intended for the thermogravimetric techniques of Duval.

One of the most startling applications of closed-loop servomechanism instrumentation is the automatic control of anesthesia described by Bickford (*10*). This instrument utilizes the brain waves from a patient to control and maintain a desired level of anesthesia. The initial effect of administering an anesthetic is to increase the activity of brain waves, but thereafter there is a progressive diminution as the degree of anesthesia deepens. In operation, the alpha-wave output of an encephalograph is fed to an integrating circuit. The pulsed output of the latter controls a stepping relay, the shaft of which is geared to the plunger of a syringe. Ether or barbiturate administration has been controlled in this way.

A band pass filter of 6 to 10 cycles has been found necessary to avoid static or 60-cycle interference. This band of 6 to 10 cycles contains all the necessary information. By means of this servosystem an even level of anesthesia has been maintained in experimental animals for 2 or 3 days! It is particularly useful in the presence of the relaxant drug, curare, the action of which invalidates most of the clinical signs upon which a skilled anesthetist depends. Additional refinements intend to supplement this equipment with a breathing indicator or combined anoxia and blood pressure indicator.

Elegant servo practice is also to be found in the precision recording refractometer of Johnson and Schnelle (*54*). It has been used as a continuous analyzer for styrene in ethylbenzene. The 5461 A. green line of an alternating current mercury arc is isolated by an interference filter and following a slit and collimater is passed through a hollow sample prism. The refraction of the beam by the sample is tracked by a photoelectric follow-up system. A folded optical system reduces space requirements and the detection system operates on the 120-cycle modulation of the arc source. No thermostats here! Temperature compensation is achieved automatically by a thermistor bridge assembly which trims the servo follow-up well enough to correct precisely for ±10° C. temperature fluctuations. For mixtures which might have widely different coefficients in their constituents, figured nonlinear networks can be computed for temperature compensation. There are many elaborate details which cannot be described here. A precision of 1 unit in the fourth decimal place is attained with accuracy and reliability. In terms of styrene content, the concentration is monitored to ±0.15%.

A semitechnical paper on the art of ruling diffraction gratings by Ingalls (*50*) is a good introduction to two articles on the latest developments in this field. The latter represent the highest order of servomechanism principle which has ever been applied to a scientific problem. Ingall's article reminds us of the enormous difficulties which have confronted the ruling-engine worker for almost a century. The newer approaches are in the direction of evaluating sources of error and devising servosystems to correct these errors continuously and automatically during the ruling operation. The commensurator, designed by Harrison and Archer (*44*), applies an old suggestion of using an interferometer as a measure of carriage displacement. By counting fringes, or fractions thereof, photoelectrically, one could use these to determine the diamond-cutting stroke and thereby become independent of residual errors in the positioning feed-screw. The commensurator does this by intercomparing two electrical wave trains, one produced by the rotation of the feed-screw, and the other by counting interference fringes, and very small fractions thereof. When a light source, using the green line from the mercury isotope of mass number 198 (Meggers arc), is used in the interferometer, the wave length under standard conditions is 5460.7532 A. The exact commensuration ratio required in the MIT dividing engine is 13.548767. This eight-figure precision ratio is achieved with a gear train supplemented by two decimal gear trains and differentials which can add or subtract small residuals. It is interesting to note the environment factors which must be considered if the interferometer-actuated servo is successfully to accomplish correction. The light source varies in wave length as the refractive index of air in the optical path varies with temperature, barometric pressure, carbon dioxide, and water vapor content. In terms of the desired precision, air pressure must be constant to 0.001 inch of mercury, air temperature to 0.01° C., partial pressure of water vapor to 0.25 mm., and of carbon dioxide to 1/100. Only pressure is held constant but the authors point out that the remaining environmental factors can be compensated automatically by well-established procedures.

A second paper, concerned with further improvements, shows how transients arise from the starting and stopping of a heavy blank carriage at the beginning and end of each ruling stroke. Harrison and Stroke (*43*) avoid this source of error by moving the blank carriage uniformly and continuously while it and the ruling diamond are kept phased together at all times by a correction servo operated from interference fringes. These are some of the elegant procedures which permit instrument manufacturers to put better gratings into that black box which the analyst calls a spectrograph or spectrophotometer.

Autotitrators are with us in abundance. They represent, in most cases, the automatic version of classical titrimetric procedures. One of many examples is chosen for illustration because it represents several unique principles. Hallikainen and Pompeo (*41*) have designed an instrument to monitor automatically the "solutizer" process for the removal of mercaptan sulfur from gasoline and thus supplement the not-too-satisfactory "doctor" test. Gasoline and a titrating solution of sodium acetate and silver nitrate in ethyl alcohol are pumped into a titration vessel, the former at a delivery rate of 45 ml. per minute and the latter at a rate which is automatically regulated to keep the combined solutions at a predetermined equivalence potential. This potential is constantly compared with a fixed reference potential and the amplified difference potential is applied in quadrature to the reagent pump motor. A special electronic feedback scheme keeps this voltage strictly proportional to the mercaptan content. Mercaptan contents from 0.000193 to 0.000855% are treated by this automatic device. If our memory serves us correctly, this essentially rheometric technique was first proposed in 1917. At that time, electronic means were not available to make the scheme practical. This may be an index to the extent to which the current state of technology determines progress.

An interesting and novel approach to the determination of salinity of sea water has been developed by Harwell and Hood (*45*). This is a radio-frequency conductance measurement without the use of electrodes in direct contact with the sample, a technique which has been highly developed in other respects. In limnological or oceanographic studies salinities can vary in ocean waters between 33.5 to 37 parts per thousand. In lagoons and estuaries values ranging between 1 to 100 parts per thousand can sometimes be found. This elegant scheme seems to suffer from one defect. A mercury thermometer is used to monitor the sample stream, and from the approximate 2% temperature coefficient of electrolytic conductance, the desired precision requires a knowledge of temperature to within ±0.001° C. It would seem that thermistor compensation or measurement would render the scheme still more useful.

There are many adjuncts to servomechanisms, and among these two examples may be selected as being typical. At the National Bureau of Standards Cadier has developed a high speed crystal clutch (*92*). This may be regarded as an alternative to the fluid magnetic clutch of Rabinow. In the crystal clutch, the output disk is located between two rotating members, a thin flexible crystal pressure plate and a heavier mounting plate or input disk. Three bimorph crystal elements, which bend when opposite potentials are applied to the crystal surfaces, are placed at 120° intervals. The exciting voltage causes the crystals to press against the pressure plate at a 1.5-inch radius and pinch the output disk between the pressure plate and the input disk. Useful torque appears at the output shaft 0.2 millisecond after voltage is applied. The observed torque is about 16.5 inch-ounces at 400 volts applied. However, the zero-voltage torque is about 7.5 inch-ounces. Rochelle salt crystals have been used, but barium titanate shows promise as a substitute material.

An exponential drive mechanism developed by Cannon (*18*) was intended to control the slit width of a single-beam infrared spectrometer. As a modification of the ball and disk integrator, the device uses a spring-loaded rotating disk driving a cylindrical roller through an intermediate twin-ball assembly. The ball cage can be displaced along a diameter of the disk, as in the conventional integrator, but in this case, the shaft of the roller is threaded and a traveling nut, engaging this thread constantly, positions the ball cage. Thus, if the ball cage is near the center of the disk, the motion transmitted to the roller is very small, but with increased motion of the latter, the ball cage is driven to constantly larger radii. The resultant output rotation is an exponential function of the disk rotation. The degree of resolution, as well as the portion of the exponential, can be selected by varying the initial displacement of the roller.

RECORDING AND CONTROLLING

Recorders and controllers are manufactured in large numbers and for almost every conceivable purpose. The American products are among the finest and most dependable in the world. Their widespread use accounts, in large measure, for the efficiency and high productive rate of our industry. Progress in this field is easily followed by the advertisements of the more prominent companies and in these, technical data on range, performance, precision, and many other useful bits of information are dependable. Adjectives and superlatives are uncommon, because this is a highly competitive business and most engineers and scientists want and get the facts. Nevertheless, there are important contributions in the literature to this subject, and a few of these may be of interest.

Although any commercial recorder can be ordered with controller attachments and very elaborate arrangements are available, there may be an occasion on which the user of a recorder may wish to add a control element. Kaufman (*57*) has described a simple adjustable fixture which can actuate a microswitch. Automatic range extension is also offered by many companies, but Webb (*110*) has described an automatic solenoid-operated switch which can introduce predetermined bias voltages to shift the range of the recorder at either end of its scale. An elegant example of this principle is described below.

An automatic thermal analysis recorder has been designed by Cunningham, Weld, and Campbell (*25*). It is an intermittent system in which a series of lines is drawn by the recorder, each line representing a set temperature and the length of the line representing the time which the sample required to change from the previous temperature. When used to study transformations in metal and alloy systems, the recorder values can be determined within 0.2° C.

An elaborate and precise means of extending the range of a recording resistance thermometer is embodied in the Leeds & Northrup Speedomax high-precision resistance thermometer recorder. A publication dealing with the design of this instrument is by Williams (*114*). The main range at any temperature is set in automatically by a Geneva movement which cuts in decade resistors. A print wheel indicates the main range which has been selected and the pen moves across the chart to provide accurate interpolation between the extremes of that range. Over the entire range, one has an effective chart width of 75 feet! To do this without loss of precision involves several subtle tricks in the way of compensating small errors. Among other things, there is automatic compensation for the effect of humidity on the chart paper. Errors as high as 0.5% could arise, but these are automatically eliminated. For the proper resistance element which is measuring the temperature, this recorder will deliver the answer continuously with a precision of 0.01% or 0.003 ohm, whichever is greater.

The balancing amplifier in this recorder embodies many of the advanced design features previously described by the author and his associates (*113*).

The potentialities of this type of instrument were pointed out some 6 years earlier by Stull (*104*, *105*), who showed its usefulness in the examination of many organic compounds in the 98 to 100% purity range. Upon cooling, a recorder draws the time-temperature curve showing supercooling and the freezing point. The freezing point of the pure material and the mole fraction of impurity are readily calculated from the curve. The instrument is equally valuable in ebullimetric, vapor pressure, calorimetric, and specific heat studies and in determining heats of reaction.

The intentional introduction of "jitter" into D'Arsonval movements such as meters, galvanometers, and recording milliameters has proved its value for some years. A definitive treatment of the subject is given by Ives (*52*). He has indicated that relay oscillators, for this purpose, with a frequency of 5 to 15 cycles, are so successful that they are being incorporated in all modern field meteorological instruments. However, their life is too short. Even with a good telephone-type relay, which is dependable for 10,000,000 operations, operation at 10 cycles uses up the dependable life in about 11.6 days (24 hours). By using a 10-cycle multivibrator driving cathode follower for output, the useful tube life runs to 40 days. With special "red-tubes" (10,000 hours) monthly checking is advisable and tube replacement is indicated every 13 months. His studies also show that jitter frequencies of less than 5 cycles require excessive amplitude to be effective and are likely to inject sawteeth into the record. Frequencies much higher than 15 cycles interfere with ink flow in some pens, converting the recorder into an efficient ink thrower! Rates above 400 cycles make continuous excavations in the chart.

The digital presentation of data is a technique which may well characterize instruments of the future. Not only can the data be printed out on tape to any justifiable number of digits, but in this form they are ideally suited for acceptance by computers and tape or card punchers. The digital recorder Series 1550 of the Berkeley Scientific Division of Beckman Instruments (*8*) is a good example of this class of recorder. A decimal scaler receives input pulses and, at a predetermined command, the information is presented in visual decimal form. A stepping switch then scans the information in each decimal counting unit in turn and applies the information through buffers to the relay matrix. When the scanning is completed, a print command is given to the printing recorder. There are automatic clearing circuits and means for switching the system back for a new count. The normal complete printing cycle is 0.8 second. Any phenomenon which can be converted to pulses can be handled in this way. Thus rotational speed can be measured by photoelectrically generated pulses and the speed can be printed on tape at very short intervals. The applications to nuclear counting are obvious.

Cathode ray delineation and recording are absolutely indispensable in electronics research. They are also widely used in almost every other field of research. Twenty years ago, Müller and Garman (*79*) demonstrated a high speed, cathode ray indicating spectrophotometer and a year later presented motion pictures delineating the kinetics of the hemoglobin-oxyhemoglobin conversion. An elegant version of this instrument has appeared after 18 years and it is manufactured by the American Optical Co. A good description of this instrument has been given by Beitz (*7*). Despite the excellence and utility of this instrument, there seems to be little or no demand for it, as far as the reviewer can learn. Perhaps, no one wishes to see so many absorption spectra in so little time.

Chance (*19*) has made many important biochemical studies with related techniques and, in the process, has made valuable instrumental contributions.

The Quantometer, or direct-reading spectrograph, has revolutionized routine spectrographic analysis. It utilizes separate photomultipliers to scan a dozen or so selected lines and compare them automatically with the intensity of a reference matrix line. A new and fascinating approach to this problem is described by Brehm and Fassel (*13*), wherein a single photomultiplier reads lines sequentially in a rapid scanning spectrometer. Elaborate electronic circuitry presents the lines on a 'scope. The pulse heights are "read" and converted to equal amplitude bursts of pulses whose number is proportional to the magnitude of the original spectral line pulse. Lines which are of interest are sorted by electronic gates. The time integrals of the spectral line intensities are computed and intensity ratios are automatically calculated after the exposure and recorded on a strip chart recorder calibrated directly in per cent. An analytical precision within 1% is attained.

A new and promising approach to tape recording made by Skellet, Leveridge, and Gratian (*98*) uses standard magnetic tape which is run over the neck of a special miniature cathode ray tube, causing an electron beam to deflect alternately between two collecting plates at the recorded audio rate, so that the current output of the tube is linearly porportional to the magnetization on the tape. The new recording head gives an average output of the tube of the order of 200 mv. compared with 10 mv. for a conventional head, and the frequency response is very nearly flat, in sharp contrast to the conventional head. A drop at high frequencies occurs which can be compensated by a simple filter section. It is ideally suited to very low frequencies, direct current levels, or pulses without distortion. The old type requires a dithering head or frequency modulation. It also shows great promise for perpendicular recording involving magnetization through the tape rather than longitudinal recording.

COMPUTERS AND DATA ASSIMILATION

Some topics in this review have indicated that the computation of data and their rapid assimilation are an intimate part of the whole problem. One striking characteristic of modern instrumental development is the increased speed with which information can be obtained from a system. No one is willing to put up with less information; the more the better. However, in many instances, the accumulated information can reach embarrassing proportions and it may well take more time to interpret the data than to obtain them.

Automatic computing and data assimilation is a vast and highly developed subject. For the immediate purpose, one may dismiss the large computers which are intended for tedious and complicated mathematical problems, but data assimilation is a more pressing question. Many devices are available for storing information on tape, on magnetic drums, or magnetic cores. Data can also be typewritten or punched on paper tape or the conventional punched cards. Sorters and collating machines are also available for classifying, grouping, or evaluating such information. There are many monographs on this subject and it is hardly profitable or possible to go into the many details. Some of us, as analysts, may have even initial difficulties in approaching the subject whenever binary arithmetic is involved. Felker of the Bell Laboratories has a useful discussion of arithmetic processes for digital computers (*36*). Vacuum tube scalers and computing elements have a valid and understandable preference for operating most reliably in a binary or "all or nothing" mode. Most of us do our thinking by long association with the radix 10. Technical progress helps us, nevertheless, because there are many automatic binary to decimal converters. The instrument designer does pay close attention to computing devices, for the simple reason that many mathematical functions can be introduced into the instrument design. It is important, in this connection, to note that a computer is not necessarily a separate or costly device.

SPECIAL TECHNIQUES AND MATERIALS

Microburet techniques, including motor-driven systems, have replaced conventional burets in many cases. A very convenient form and its uses is described by Lazarow (*63*). Applications in which it is used with Beckman spectrophotometer cells are mentioned by Lowry and Bessey (*67*). The work of DeFord on the external coulometric generation of reagents (*29*) is only the beginning of what may be expected in "electrical" characterization of volumetric analysis. Excellent notes and suggestions on the design and calibration of spectrophotometers are to be found in the paper by Normand and Kay (*83*).

A symposium on polarization phenomena yielded several papers which summarize developments up to 1951 on a large variety of topics. A complete summary of 25 years of developments on synthetic polarizers is given by Land (*61*). West and Jones (*112*) discuss polarization elements as used in optical instruments. West and Jones (*111*) in another paper discuss sinusoidal modulators. The latter may be defined as two polarizers rotating at substantially constant relative velocity. Their possible use in the development of optical servo systems is discussed by these authors. The paper by Brode (*16*) contributes nothing instrumentwise, but is a valuable résumé by a noted authority on the relationship between optical activity and the configuration of asymmetric organic compounds.

Discussions of stellar photometry would seem far afield from the analyst's interests but the semitechnical discussion by the renowned astronomer, Joel Stebbins (*103*), contains much intriguing information. After all, the astronomers were the first to note and make use of the fact that refrigeration of a phototube greatly increases the signal to noise ratio. Recent techniques utilizing a rotating pinhole enable astronomers to photometer stars in broad daylight! Conversely, since the secular variation of brightness is known for some stars, the method may be used for studying the variation in atmospheric haze. Some of this entails chemical analysis "way upstairs."

A new, magnetically hard material, which is given the name Ferroxdure, has a large coercive force with a low saturation magnetization (*89*). It has the approximate composition of $BaFe_{12}O_{19}$. Its properties result in a large resistance to demagnetization. Its uses are in permanent magnets, coupling devices, and focusing coils.

A useful compilation for work in ultraviolet and infrared spectrophotometry lists the infrared absorption and ultraviolet cutoff for a dozen important solvents. This general discussion of solvents by Anderson and Steward (*2*) includes a chart summarizing the data. Copies may be obtained free of charge from Eastman Organic Chemicals Department, Distillation Products Industries, Rochester 2, N. Y.

Taylor (*106*) describes a technique and instrument for maintaining highly regulated low voltages at rather heavy currents for electrodeposition and coulometry. A relaxation oscillator of controllable period feeds pulses to a power amplifier and rectifier stage. The output voltage is balanced against a reference voltage and the resultant error signal is fed back regeneratively to control the pulse repetition rate.

The techniques of modern scintillation counting are described in a monograph by Berks (*9*). This deals with all the modern photomultipliers and all of the scintillators, organic and inorganic, which are suited for the purpose.

Chromatographic scanners of almost every conceivable type have been described and several commercial instruments are available. Laurence (*62*) has described one particularly suited to the scanning of electrophoretic patterns.

The standards of illumination as revised frequently by the C.I.E. (Commission Internationale de l'Eclairage) serve to define the standard illuminants A, B, and C. The latest recommendations are in a report by Davis, Gibson, and Haupt (*28*). These specify the filter solutions and all related factors which with a tungsten lamp will produce exactly the colors corresponding to color temperatures of 2848°, 4800°, and 6500° K. The new set of data are in tabular form at 10 mμ intervals from 300 mμ in the near ultraviolet to 780 mμ in the red.

The field-ion microscope of Erwin Müller utilizes cold emission under high gradients whereby electrons are directed to a fluorescent screen which is concentric with the source. A recent paper by Müller (*75*) shows that adsorbed ions can be pulled off as positive ions from the tip of the object by reversing the polarity. Rapid feeding of adsorbed atoms can secure a continuous image of the tip.

Essentially television techniques are applied in the flying-spot microscope (*35*). This British development uses a cathode ray tube as a light source. An unmodulated 405 line scanning roster moves the cathode ray spot and by straightforward optical projection is made to scan a very small area of the specimen. The light which leaves the microscope is picked up by a photomultiplier and the amplified output is presented on a large cathode ray tube (picture tube). The sweep rates on the latter are synchronized with the scanning tube, so that the image on the viewing screen is a faithful copy of the specimen scanned. The advantages are: ability to present a view to a large audience, to minimize heating of the specimen, to use near ultraviolet to increase contrast, and to vary contrast within wide limits by purely electrical means. Similar schemes have been used to count cells automatically with gating and amplitude criteria to correct for clumping or aggregation of the cells.

The production of photoconductive lead telluride cells has been improved by a technique for sealing on synthetic sapphire windows. The latter are transparent out to 6 microns. In this technique (*20*) a highly polished borosilicate glass ring is premelted on the sapphire plate at 900° C. in order to wet it. The temperature is reduced to 780° C. and the graded seal from the photocell envelope is then pressed onto the glass ring. The assembly is annealed at 470° C. An appendix to this article, by Young, shows how windows of synthetic periclose (MgO) can be sealed on. These windows at 1-mm. thickness give 40% transmitting at 9 microns. They will withstand repeated heating to 500° C. with no failure in the vacuum seal. Improvements in technique use radio-frequency heating.

The lowly art of soldering has many tricks and special techniques of use to the experimentalist. An excellent résumé of some of the latest methods is given by Pugh (*91*).

Thirteen references to the use of organosilicon coatings on glass are listed by Gilbert (*37*). The water-repellent action of these coatings is of great use in glass electrodes, absorption cells, and pipets.

A mercury jet switch has been invented (*46*) which can perform 1800 switching operations per second at a noise level of only 20 mv. across a 200-ohm load. It has been applied in a temperature mapping and monitoring system wherein 49 temperatures are repeatedly monitored every tenth of a second.

SUPPLEMENTARY TOPICS

A few contributions are described here briefly for their obvious connection with instrumental developments, despite the fact that they may be described more fully in other review articles of this series.

Chemical analysis by neutron spectroscopy is discussed by Taylor, Anderson, and Havens (*107*) in a paper with 29 references. It is a good summary of prevailing techniques in this field.

A good introduction to the techniques of nuclear magnetic resonance spectrometry is given by Malling (*69*). The emphasis here is more on the instrumental aspects, although the elementary theory is discussed.

Gas-liquid partition chromatography is an important subject and the extraordinary separations which can be achieved have led to the development of at least a half dozen commercial instruments. The earliest efforts in this direction by James and Martin (*11*) mark the beginning of this rapidly advancing technique. The subject matter and techniques belong to the general field of chromatography.

Counting of individual cells on a fluorometric basis and using television and electronic scanning methods are described by the Sloan-Kettering group (*99*).

The analyst is likely to associate nuclear techniques almost exclusively with the use of tracers. Although the latter are of enormous and continuing value, there is much more to be learned from them than this. Neutron spectroscopy, activation analysis, and many other approaches seem to offer far more subtle possibilities. An outstanding accomplishment is embodied in the work of Smyth and Otvos (*101*) in studying the absorption of beta particles. Their research has led to the development of two commercially available instruments for the accurate determination of hydrogen, or of the hydrogen-carbon ratio in hydrocarbons to a precision of 0.1%. This can be done in a few minutes if the density of the liquid is determined to a comparable degree of precision.

For the past 3.5 years, the reviewer has made a systematic study of the backscattering of beta particles by matter. By late 1953 this had been extended to 32 elements and some 40 compounds (*77*). Several hundred other systems have been studied since then and it is now possible to state general conclusions for all atoms and molecules. The relative backscattering is a discontinuous function of the atomic number Z, but linear in Z within each period of the periodic system. The discontinuities arise at each rare gas, terminating the period. The scattering of all compounds is accurately predicted from the intrinsic scattering of their constituent atoms and known weight fraction. Isomers scatter identically, but differ in absorption. Organic compounds scatter in completely predictable fashion, but hydrogen shows an anomaly, which fortunately is directly proportional to its weight fraction. Crystalline substances of high purity can be checked within 0.07% of the predicted scattering.

The ultracentrifuge has been an indispensable tool in studying macromolecules for more than 30 years. In this connection an account of the most recent advances in ultracentrifuge design, by the greatest living authority on the subject, is of more than passing interest. Beams (*6*) discusses the circuitry involved in magnetic suspension ultracentrifuges. Accelerations of 515,000,000 times gravity have been achieved. With his special circuits, constancy of rotational speed to 1 part in 10^8 can be attained.

A forward scattering photometer for the measurement of airborne contaminants or aerosols has been described by Sinclair (*97*). A concentration range of 1 to 100,000 can be covered by a logarithmic photometer circuit or more precisely in a limited range by a linear amplifier.

A gas detector, based upon stoichiometric reaction of certain gases with mercuric oxide and subsequent photometry of the mercury vapor is manufactured by Taller and Cooper, Brooklyn 71, N. Y. It is based upon the fundamental studies of McCullough (*68*).

The layman has had an opportunity to learn that a wide assortment of automatic instruments can serve to present continuous clinical information (*65*).

There is no apparent end to cooperative and comparative tests of instrument performance. One of these involved 15 cooperating laboratories in checking 12 Cary recording spectrophotometers and 11 Beckman DU instruments (*17*). In this reviewer's opinion tests of this kind usually indicate that Beckman and Cary have known what they were doing right along.

Volumes could be written on the performance of instruments, their ruggedness, need for recalibration, servicing, etc. Some interesting data came from the Navy as a result of careful studies on radar failure (*34*). Arranged in order of "down-time" hours they turned out to be (1) mechanical, (2) electrical, and (3) electronic. Corresponding data on analytical instruments would be interesting, although environmental factors might be different.

CONCLUSIONS

Analytical chemistry is now drawing heavily on instrumental developments in every field of science and technology. There have been assertions that instrumental analysis is nothing new; that it is as old as analysis itself. That can be said with confidence only by one who is completely unfamiliar with modern instrumentation. It is only within a few years that the trends have deviated from the mere improvement of classical techniques. Some of the newer approaches have no counterpart in classical analytical chemistry.

The National Bureau of Standards has always been of inestimable aid to the analyst as well as to other scientists. It has also always had heavy responsibilities and has maintained invaluable services (*48*). Seven years ago, E. U. Condon, then director of the bureau, asked the question, "Is there a science of instrumentation?" (*23*). The reviewer had maintained that there is such a science, at least 15 years before that, but it is always useful to have someone of importance raise questions of this sort, and moreover, to do something about it. Shortly thereafter, the bureau established its Office of Basic Instrumentation under the able direction of W. S. Wildhack. We need more of this sort of thing, and the effort and resources for the purpose could well be enhanced manyfold for an enterprise as important as instrumentation.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Electroanalysis

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THIS review attempts to survey the developments in the fields of electroanalysis and coulometric titrations since the publication of the last review in this series (*29*). As in the previous reviews, only a few selected papers in the field of fundamental electrochemistry are included. However, most of the significant developments in this field have been very adequately reviewed elsewhere.

BOOKS AND REVIEW ARTICLES

Delahay's treatise (*32*) on new instrumental methods in electrochemistry presents the detailed theoretical background as well as the more practical aspects of a variety of electroanalytical methods. The principles and applications of electroanalytical methods have been reviewed by Smelik (*140*). Meites (*101*) has included a brief discussion of controlled potential electrolysis in his recent book on polarographic techniques. Reviews of specific types of electroanalytical methods are mentioned later under the topics to which they pertain.

THEORY

Recent experimental results and theoretical developments in the study of the kinetics and mechanism of electrode processes have been reviewed in considerable detail by Bockris (*10, 11*) and Grahame (*56*). Grahame has also included a summary of recent work pertaining to the electrical double layer. Anyone working in the field of electroanalysis will find these reviews of recent fundamental developments invaluable. Two papers by Gerischer and Vielstich (*53, 155*) pertaining to the kinetics of the deposition of both simple and complex metal ions were published too recently to be included in the reviews cited.

Studies on electrodeposition from very dilute solutions are of interest not only because of their direct application in trace analysis but also because they shed light on the fundamental nature of electrodeposition processes. Work in this field has been summarized in a very recent review by Rogers (*126*) and is not discussed here.

ELECTROSEPARATIONS

Mercury Cathode Separations. Bagshawe and his associates (*3*) have made further studies on the separation of large amounts of elements such as iron, chromium, and nickel in ferrous alloys at a mercury cathode as a preliminary step in the determination of minor elements such as aluminum, titanium, zirconium, and vanadium. Quantitative removal of chromium is possible to achieve but only with considerable difficulty. Certain alloy steels, particularly those containing tungsten, molybdenum, niobium, or large amounts of chromium, present many difficulties.

For the determination of cadmium and zinc Nakano and coworkers (*108*) recommend deposition of the cadmium into a mercury cathode, where it may be determined by measuring the electrical resistance of the amalgam; the zinc remaining in the aqueous solution may then be determined titrimetrically. Meites (*98*) has recommended the use of controlled potential electrolysis at a mercury cathode for the removal of traces of heavy metal ions from salts that are to be used as supporting electrolytes in polarographic analysis. This technique should be useful in many other cases for freeing reagents from traces of heavy metals.

The quantitative deposition of manganese at a mercury cathode has proved to be difficult with the usual types of electrolysis cells. One solution to this problem has been offered by Coriou and his coworkers (*22*). They found that manganese could be deposited quantitatively by using the constantly renewed mercury cathode developed several years ago (*23*). Mercury dropping slowly from a capillary tube is added continuously to the small, shallow, mercury cathode, which is maintained constant in volume through the use of an overflow device. In this manner the manganese amalgam is continuously removed from the contact with the aqueous phase, thus minimizing redissolution of the manganese. Lazarevic (*83*) has proposed an alternative arrangement in which the cathode consists of a film of mercury which flows continuously down the surface of a wire and thence to a receiver outside the electrolysis cell. From the viewpoints of efficiency and speed this arrangement was claimed to be superior to that of Coriou.

Still another type of renewable mercury cathode cell, designed particularly for the deposition of the alkali metals, has been described by Oka and his associates (*112*). This cell consists of a glass tube, open at both ends, supported vertically in a beaker with its lower end a few millimeters from the bottom of the beaker. Mercury is placed in the beaker to a level well above the lower end of the central tube. Water is then added to the central tube and the solution to be electrolyzed is placed in the annular space between the tube and the beaker wall. Even with vigorous stirring this arrangement prevents transfer of the aqueous phases between the two compartments, but the mercury moves readily between the two. Hence sodium amalgam formed in the external compartment moves into the inner compartment, where it reacts with the water to form sodium hydroxide, which may be determined readily by titration with acid. In this manner milligram quantities of sodium may be transferred quantitatively from the outer to the inner compartment. Operation with a controlled cathode potential permits a quantitative separation of sodium from potassium. It would appear that cells of this type could be used to advantage in the separation of other active metals.

Small amounts of europium may be quantitatively removed from samarium by deposition into a mercury cathode from lithium citrate solution (*115*). A small percentage of the samarium is deposited also. Because a very large excess of lithium is present in the aqueous phase, the potential of the cathode is that of a lithium amalgam electrode, of nearly constant composition, in contact with an aqueous lithium solution of essentially constant composition. Hence, this separation is in essence a controlled potential separation, even though no external potential control is employed.

Schmidt and Bricker (*133*) have reported that microgram quantities of copper, lead, cadmium, cobalt, nickel, and iron may be separated from several grams of vanadium by deposition in a mercury cathode. All these metals except cadmium may then be determined by removal of the mercury by distillation, followed by polarographic analysis of the residue in essentially the same fashion as was reported earlier for the determination of impurities in uranium compounds (*19*). Recovery of cadmium is low because some cadmium distills with the mercury. In order to avoid this loss Radak (*122*) recommends that the metals be recovered by washing the amalgam with 5% nitric acid. Schmidt and Bricker (*133*), however, report that the use of chemical oxidizing agents is unsatisfactory; they prefer the anodic stripping technique described below.

Copper, cadmium, lead, zinc, and thallium may be recovered from dilute amalgams by electrolytic stripping at controlled potential (*24, 78, 120, 133*). On the other hand, the passive metals—iron, cobalt, and nickel—and also bismuth cannot be stripped without simultaneous oxidation of the mercury. For this reason controlled potential depositions into a mercury cathode followed by controlled potential stripping of the amalgams permit several separations—e.g., iron from zinc—which are not possible with electrodeposition alone. Scacciati and D'Este (*132*) found that trace amounts of zinc in cadmium could be determined by dissolving the sample in mercury, followed by quantitative electrolytic stripping of the zinc into sulfuric acid solution. Under proper conditions very little cadmium is oxidized, and thus a polarographic determination of the zinc is feasible. Hickling and Maxwell (*63*), as well as several of the authors cited above, have treated some of the theoretical aspects of the anodic dissolution of amalgams.

Although they have no direct analytical applications, the studies of Lihl and Jenitschek (*86*) on the simultaneous deposition of two or more metals into mercury are of interest. Likewise the polarographic results obtained by Rosie and Cooke (*127*) with mercury pool electrodes in stirred solutions will be of interest to anyone concerned with mercury cathode separations.

Controlled Potential Separations. Despite the fact that several excellent potentiostats have been described in the earlier literature and that these items of equipment are now available commercially, reports of new instrumentation for automatic control the electrode potential continue to appear (*65, 68, 69, 72, 111, 112*). None of these instruments seems to offer significant advantages over others described earlier. A special-purpose potentiostat with a regulation of ±0.5 mv. for currents up to 30 ma. has been described (*134*). Vielstich and Gerischer (*155*) have described a potentiostat with a very fast response time; this instrument was designed particularly for the study of transient phenomena.

Ishibashi and Fujinaga (*66, 70*) have found that copper can be separated from bismuth by deposition at −0.30 volt *vs.* S.C.E. from a solution containing tartrate, succinate, and hydrazine buffered at pH 5.8. After separation of the copper, bismuth can be removed at −0.40 volt. Lead, antimony, arsenic, tin, cadmium, and zinc do not interfere. For the determination of small amounts of bismuth, lead, and cadmium in the presence of much copper, removal of the copper by the method outlined above followed by polarographic determination of the minor elements was recommended (*67*). Diehl and Craig (*34*) separated copper from cadmium in ammoniacal solution by controlling the cathode potential at −0.73 volt *vs.* S.C.E.; this separation is useful in the analysis of silver solder. According to Aylward and Bryson (*2*), copper may be separated from lead at a potential of −0.35 volt using a phosphoric acid electrolyte. Lead can be deposited after the copper, and then tin, if present, may be determined in the spent electrolyte by titrimetric methods. Oka and coworkers (*113*) have described a method for the deposition of copper from hydrochloric acid solution containing hydrazine, but their results are less satisfactory than those obtained by other methods. Attempts to effect quantitative deposition of tin were unsuccessful.

Lindsey and Tucker (*87*) have given directions for the determination, on a micro scale, of copper, lead, and zinc in brasses and bronzes. With a few modifications the method can be adapted to the determination of antimony, copper, lead, and zinc in white metals and solders (*88*). According to Dean and Reynolds (*28*) bismuth, together with copper, may be deposited at −0.3 volt from a hot (80°C.) solution containing citrate and hydrazine and buffered at pH 3.0. After addition of concentrated hydrochloric acid, antimony may be plated at the same potential. Finally, tin, together with lead, may be deposited from the same solution at −0.65 volt. Methods for the separation of the platinum metals have been devised by Bubernak (*15*).

Internal Electrolysis. Internal electrolytic methods, in particular the application of these methods to the determination of small amounts of impurities in metals and alloys, have been reviewed by Giordani and coworkers (*54*). These authors recommend the use of an electrolysis cell which permits continuous renewal of the anolyte, thus assuring a more nearly constant potential throughout the electrolysis. It is stated that this technique has a higher separation efficiency than conventional methods. Procedures for the separation of copper, cadmium, and tin are given.

Rubio Felipe (*130*) stated that small amounts of copper in steel may be determined by plating onto a platinum cathode from acidic solutions containing hydrazine sulfate; a platinum anode and a ferrous sulfate–hydrazine sulfate anolyte were used. Serdyuk and Barash (*137*) reported a method for the separation of nickel from manganese using ammoniacal oxalate solutions. Sommer (*141*) has described procedures for the microdetermination of gold and silver. Okac and Sommer (*114*) have worked out the optimum conditions for the determination of bismuth, antimony, and copper, alone and in the presence of lead, nickel, copper, and iron. Lead or aluminum anodes coated with a film of collodion were used. The method outlined is stated to be capable of determining copper with an accuracy within ±1% in ordinary steel, but it fails for alloy steels. Schmidt and

Bricker (*133*) have pointed out that the use of internal electrolysis obviates the need for a potentiostat in carrying out controlled potential oxidation of amalgams.

Constant Current Separations and Electrogravimetry. According to Bommer (*12*), a roughened platinum plate anode is more satisfactory than the conventional gauze electrode for the deposition of lead as the dioxide; the deposit adheres much more firmly to the former. Umland and Kirchner (*153*) report that the optimum concentration of nitric acid for the deposition of lead dioxide lies between 5 and 9% on a weight basis. Iron interferes with the deposition if present in amounts exceeding half of the weight of lead present. Nicolas (*109*) has reported a method for determining lead in steel by cathodic deposition from hydrochloric acid–hydroxylamine electrolyte.

According to Foschini (*44*) it is unnecessary to plate the platinum cathode with copper before depositing tin or to add oxalate to the electrolyte in order to obtain satisfactory deposits. Sakuraba (*131*) states that it is possible to determine molybdenum by adding a known weight of copper to the sample and to deposit the molybdenum (as $MoO.3H_2O$) and the added copper simultaneously on the cathode. Iron may be deposited quantitatively from oxalate solutions; however, since related elements such as cobalt and nickel are deposited along with iron, it has not been possible to devise a practical electrogravimetric method for the determination of iron (*82*).

Tschanun (*148*) has further investigated the microdetermination of copper and zinc in brasses and bronzes. The British Standards Institution (*14*) has published specifications for micro scale electrolysis apparatus.

Whitson and Kwasnoski (*157*) employed electrodeposition onto a nickel disk for the recovery of minute traces of uranium from urine after first decomposing the sample by nitric acid digestion, evaporation, and ignition. Ballentine and Burford (*6*) determined radioactive cobalt in biological materials by wet-ashing the sample, separating cobalt as cobaltic hydroxide, and finally plating cobalt metal from a buffered fluoborate solution. The deposited cobalt was then determined by a measurement of its radioactivity.

Takagi and Hirano (*145*) reported that the application of ultrasonic waves markedly decreased the time required for the cathodic deposition of copper; the increased speed of deposition was thought to be due to the strong stirring action produced at the electrode surface.

The use of perchloric acid in electrochemical analysis has been reviewed by Norwitz (*110*); in many cases perchloric acid is to be preferred over sulfuric acid, both for dissolution of the sample and for adjusting the acidity of the final solution.

ELECTRO-OXIDATION AND REDUCTION

Electrolytic Dissolution and Decomposition. Further studies on the applicability of the electrolytic method in the analysis of inclusions in steels have been made by Papier (*116*) and by Šícha (*139*).

Thoburn (*146*) was able to decompose organic boron compounds by electrolysis in nitric acid solution between platinum electrodes as a preliminary step in the titrimetric determination of boron in these compounds. Despite the attractive possibilities of the electrolytic decomposition method, no other applications of the method have been reported in the past 2 years. Elving (*41*) has given a comprehensive report on the electrochemical fission of carbon-halogen bonds and has pointed out the applicability of polarography to the determination of organic halogen compounds. It would appear that electrochemical decomposition of such compounds could replace the usual Carius and peroxide fusion methods in some cases as a preliminary step to the titrimetric or gravimetric determination of halogens. In some cases it would appear that selective decomposition could be achieved, particularly if the electrode potential is controlled, thus permitting the determination of individual halogenated compounds in mixtures. There seems to be no doubt that electrolytic decomposition methods are deserving of greater attention and exploitation than they have received in the past.

A number of fundamental studies on glow-discharge electrolysis and alternating current electrolysis are of interest in that they suggest possible analytical applications. Several authors (*13, 27, 62, 76, 106*) have shown that hydroxyl free radicals are formed when a glow discharge is set up between a solution of an electrolyte and an external electrode. Such radicals are powerful oxidizing agents. Under optimum conditions Hickling and Linacre (*62*) found that seven equivalents of ferrous iron could be oxidized to the ferric state for each faraday of electricity used. In the absence of oxidizable substances the hydroxyl radicals dimerize to form hydrogen peroxide; in solutions which are more than 10*M* in sulfuric acid, peroxysulfuric acid rather than hydrogen peroxide is the main product (*106*). Likewise when aqueous solutions of sulfuric acid are electrolyzed with low frequency alternating current between platinum electrodes, both of which are in the solution, significant quantities of hydrogen peroxide are produced (*71*). In this case also the formation of hydrogen peroxide is thought to arise from OH or HO_2 radicals which are formed at the electrodes. No application of such methods to the oxidation of organic compounds has come to the attention of the writer.

Galfayan (*50*) has suggested the use of electrolytic methods to effect reduction of iron and titanium to the ferrous and titanous states, respectively, as a preliminary step to their determination by standard titrimetric methods.

Electrographic Analysis. No significant developments of general interest in the field of electrography have appeared, but several new applications and specialized techniques have been described. Applications to alloys in general (*97*), to ferrous alloys (*43*), and to nonferrous alloys (*55*) have been discussed. One paper has been devoted to an extensive discussion of the uses of electrography in the examination of uranium minerals and ores (*20*) and another (*57*) to the detection of metal ions in sulfide ores. Structure etching of minerals and the identification of a variety of metal ions in ores have also been treated (*58*).

COULOMETRIC TITRATIONS

Interest in the coulometric method continues to increase. Many new techniques, applications, and items of equipment have been described in the past 2 years. The principles and the applicability of the method have been reviewed by Bishop (*9*), Cooke (*21*), DeFord (*30*), Delahay (*32*), Furman (*48*), Gauguin (*51*), Kimoto (*75*), Reilley (*123*), and Smelik (*140*). The applicability of polarization curves (*7*) and of current-scan polarography (*1*) in establishing the feasibility of and the optimum conditions for coulometric analyses has been pointed out. Eckfeldt and Kuczynski (*39*) have reiterated the pleas made earlier by others for an increased utilization of coulometry as a substitute for chemical primary standards. They point out that electrical standards are more reliable than chemical standards, and that modern instrumentation makes the measurement of coulombs a very accurate and simple operation. Craig and Hoffman (*25*) have redetermined the value of the faraday constant by the electrolytic oxidation of Bureau of Standards certified sodium oxalate. A mean value of 96,492 ± 3 absolute coulombs per equivalent (chemical scale) was found for this constant. Reilley (*123*) has pointed out the need for including a discussion of coulometric titrations in instrumental analysis courses and has given directions for a suitable student experiment.

Primary Processes. The significant effects of electrode preparation and of trace impurities in the solution on the course of controlled potential electrolyses have been pointed out by Baker and MacNevin (*4, 5*). A method for the successive determination of nickel and cobalt by controlled potential coulometric analysis with a mercury cathode has been detailed by

Lingane and Page (*93*). Meites (*99*) has suggested the use of coulometric analysis in conjunction with polarography for the simultaneous determination of two substances which are reduced at nearly the same potential. This method is applicable only when the ratio of the diffusion coefficients of the two species is about 1.5 or greater. Coulometric methods for the assay of trichloroacetic acid in the presence of dichloroacetic acid (*103*) and for the determination of picric acid (*102*) have been described by Meites.

Tur'yan (*149*) has found that a number of nitro compounds can be reduced with 100% current efficiency when the potential of the cathode is controlled at a suitable value. Tur'yan employed an electrolysis cell with a separated anode compartment where iodide ion was oxidized to iodine during the electrolysis. The number of coulombs was then measured by titration of the liberated iodine, making a separate coulometer unnecessary. Sease (*136*) has also described coulometric methods for the determination of nitro compounds. Marple and Rogers (*96*) have employed the principle previously developed for the determination of silver (*94*) for carrying out coulometric determinations of lead in concentrations as low as $10^{-8}M$. Yamada (*159*) has been able to determine 1 to 100 γ of copper by controlled potential electrodeposition; a mercury microcoulometer (*160*) was employed to measure the quantity of electricity required. The method was found suitable for the determination of traces of copper separated by paper chromatography. Thwaites and Hoare (*147*) have reported their experiences with the anodic stripping method developed by Kumze and Willey (*80*) for the determination of tin and of tin-iron alloy in electrotinplate. Waite (*156*) has reported very satisfactory results on the determination of the thickness of copper, nickel, zinc, and cadmium coatings on steel, of chromium and nickel on copper, and of chromium on copper by the anodic dissolution method. A commercially available instrument (Kocour Co., 4800 South St. Louis Ave., Chicago 32, Ill.), based upon the design of Francis (*45*), was employed for these tests.

Lewartowicz (*85*) has proposed a novel coulometric method for the determination of soluble oxidizing agents which have soluble reduced forms. If the system is reversible, dE/dQ (where E is the potential of the system and Q is the quantity of electricity used) is a minimum when exactly one half of the oxidizing agent has been reduced; from the position of this minimum it is then possible to calculate the quantity of oxidizing agent originally present. No actual examples of analysis by this method have been reported, but it would appear that precision would be poor.

Secondary Processes. Perhaps the most significant development in coulometric titrimetry in the past few years has been the announcement by Meyer and Boyd (*104*) of a method for the electrolytic generation of Karl Fischer reagent for the determination of water. This method has an absolute sensitivity of about 2 γ of water and a precision within 1 to 2%. Although the method has some drawbacks, in particular a requirement of a small supplementary generation current to compensate for decomposition of the reagent, it can undoubtedly be further refined and should find widespread utility.

The halogens continue to find increased uses as coulometric intermediates. Iodine has been used for the determination of sulfides (*121*), thiosulfate (*128*), and selenium (*129*). Further applications of bromine in the determination of phenols (*154*), sulfanilimides (*144*), and salicylic acid (*73*) have been reported. Another report (*81*) by Landsberg and Escher on the use of the Titrilog, which employs electrolytically generated bromine, for the determination of sulfur compounds in gases has appeared. Chlorine, generated electrolytically from a solution of 0.2 to $1.2M$ hydrochloric acid in 80 to 90% acetic acid, has been found a satisfactory reagent for the determination of unsaturation in long-chain fatty acids (*26*). Procedures for the external generation of all three halogens have been described (*119*).

Procedures for the determination of ferrocyanide (*35*) and mixtures of iron and titanium (*36*) with ceric ion have been given. Tutundzhic and Mladenovic (*151*, *152*) have reported suitable conditions for the generation of what they believed to be permanganate ion with 100% current efficiency by electrolytic oxidation of manganous ion and have used this ion for the determination of iron, arsenite, and oxalate. Horn (*64*) independently made a similar study, but he concluded that the oxidizing intermediate was manganic ion rather than permanganate. Ferricyanide has been used successfully for the oxidimetric titration of thallous thallium (*60*). The use of a mercury pool for the generation of titanous ion by the electrolytic reduction of titanic ion is superior to previous procedures using a gold cathode (*117*). Titanous ion can also be generated successfully at a small mercury pool in an external generation cell; titanous ion so prepared has been used successfully for the titration of a number of organic dyestuffs (*116*). Malmstadt and Roberts (*95*) determined small quantities of vanadium in titanium tetrachloride by hydrolyzing the sample and then titrating the vanadate with titanous ion formed by electrolytic reduction of the titanic ion in the sample. These authors were able to obtain 100% current efficiency in the reduction of titanic ion at either titanium or platinum electrodes.

Shults, Thomason, and Kelley (*138*) have found that uranium(IV) can be generated with 100% current efficiency at a platinum electrode from solutions of uranyl sulfate in dilute sulfuric acid. This moderately strong reducing intermediate was used for the determination of oxidizing agents such as ceric and dichromate ions. Dunham and Farrington (*37*) have used metallic copper as a reductimetric reagent. An excess of copper is plated onto a platinum electrode from a cuprous halide solution, the sample of oxidant is added, and, after sufficient time has been allowed for the reaction to go to completion, the excess copper is stripped from the electrode. The difference in the number of coulombs required for plating and for stripping is a measure of the oxidant added.

Lingane (*91*) has described methods for the automatic titration of dilute acids with internally generated hydroxyl ion. Carson and Gile (*18*) determined orthophosphate by conversion to orthophosphoric acid in an ion exchange column followed by coulometric titration of the acid so formed. Streuli (*143*) has found that hydrogen ion may be generated in acetonitrile containing lithium perchlorate ($0.05M$) and a small amount (about 0.3%) of water. Bases which are too weak to be titrated in aqueous media may be determined precisely in this solvent.

Several authors (*77*, *89*, *150*) have described procedures for the titration of halide ions with electrolytically generated silver ion. These ions may also be determined by titration with electrolytically generated mercurous ion (*64*); the latter reagent appears to be superior when the amount of halide in the sample is relatively large (about 1 mole or more). As thiourea forms a very stable complex with silver ion, it may be determined by treating the sample with an excess of ammoniacal silver bromide solution followed, after acidification, by titration of the liberated bromide ion by electrolytically generated silver ion (*107*).

Leisey (*84*) has described an automatic instrument and has outlined procedures for the determination of mercaptans by a coulometric argentometric method. Lingane and Hartley (*92*) reported that ferrocyanide can be generated successfully by electrolytic reduction of ferricyanide at a platinum cathode. This reagent was used for the titration of zinc (precipitation of $K_2Zn_3[Fe(CN)_6]_2$) in a solution buffered at pH 2.0.

Several interesting reports on the coulometric generation of gases have appeared. Button and Davies (*16*) and Kehren (*74*) have described very similar devices for studying the absorption of oxygen by various test samples. In both cases closed systems were employed and oxygen was generated electrolytically at a rate equal to its consumption by the sample, so that a constant pressure was maintained in the system. In both cases the generation current was integrated through the use of a second electrode reaction which produced a volume or a pressure of gas proportional to the amount of oxygen consumed. Hannah and his coworkers (*59*) employed a similar device for studying the effectiveness of various catalysts on the rate of recombination of hydrogen and oxygen; in this case both gases were supplied by the electrolysis of water at a rate equal to their rate of recombination. Electrolytically generated hydrogen has been used successfully for the analytical hydrogenation of a number of organic compounds (*105*); an account of this work will be published in the near future.

A very interesting account of the applicability of the coulometric method to titrations in microvolumes of solution has been given by Schreiber and Cooke (*135*). Successful titrations of microgram and submicrogram amounts of arsenic, hydrochloric acid, and sodium hydroxide in solutions having a total volume of 30 μl. were carried out. This technique should be applicable to many ultramicrotitrations.

A method for the coulometric titration of oxygen has been reported (*142*), but details were not available to the author.

Porter and Cooke (*120*) have reported that metals such as cadmium and thallium can be stripped from dilute amalgams with 100% current efficiency even though a constant electrolysis current, which exceeds the diffusion current, is used. Current which is in excess of the diffusion current causes the formation of a film of mercurous chloride on the electrode. However, when the current is interrupted the mercurous chloride oxidizes the metal in the amalgam, and thus acts as a coulometric intermediate. By carrying out the stripping with successive small increments of current and allowing the potential of the electrode to reach a constant value after the addition of each increment, very satisfactory coulometric titrations can be accomplished. The end point is indicated by a large permanent change in the electrode potential.

Apparatus. An electromechanical instrument which is capable of maintaining an electrolysis current constant to within ±0.01% has been described by Lingane (*90*). Although an instrument of this type does not respond as rapidly as an electronic regulator, its response is adequately rapid for all practical purposes. A similar instrument has been designed by Dunn, Mann, and Mosley (*38*) for another purpose, but it should function satisfactorily for use in coulometric titrations also. Gerhardt and coworkers (*52*) and Ehlers and Sease (*40*) have described modified versions of the Reilley, Furman, and Adams (*124*) electronic constant current supply. Reilley (*123*) has described a simple constant current supply designed particularly for student use. Furman and coworkers (*49*) have described a transistor power supply which will maintain a current constant to within better than ±0.1%. The author has found that a commercially available power supply, the Model M10A10 meter calibrator manufactured by Kalbfell Laboratories, San Diego, Calif., functions very satisfactorily as a constant current source.

The Beckman autotitrator continues to find application as the control instrument in automatic coulometric titrimetry (*52, 89, 91, 92, 118*). New automatic control circuits for use in conjunction with pH meters have been described by Carson (*17*) and by Bett and coworkers (*8*). Richter (*125*) has succeeded in solving the difficult problem of designing an automatic trigger for use in conjunction with a variety of amperometric indication systems. Leisey (*84*) has described an automatic instrument, employing amperometric indication, designed particularly for the titration of mercaptans; this instrument is available in modified form from the Central Scientific Co., Chicago, Ill. Bubbles and other light-scattering bodies in the solution being titrated prevent the use of conventional photometric end-point detection methods, but Wise and associates (*158*) have described a differential photometric control system which minimizes such interferences.

Because of the limitations of the constant current method, in which the number of coulombs is calculated from the current-time product, increasing attention has been devoted to the development of precise direct-reading coulometers which may be used in all types of coulometric analyses. Bett (*8*) and Parsons (*118*) and their associates have found integrating motors to be very satisfactory coulometers. These devices are simple, reasonably rugged, and usually accurate to within ±0.1% when operated under proper conditions. Coulombs are indicated directly by a revolution counter attached to the motor armature shaft. Meites (*100*) has described a coulometer which is essentially a velocity servosystem. This instrument has an accuracy which is about the same as that of an integrating motor when the latter is operated under optimum conditions. The velocity servosystem maintains its accuracy over a much wider range of currents, but it is more expensive and more complex than an integrating motor. A milliampere-hour meter will serve as a direct-reading coulometer; an accuracy within ±0.5% is claimed for currents between 10 and 30 ma. (*61*). Kramer and Fischer (*79*) have described a simple electronic coulometer which is said to be accurate to within ±1% over a wide range of currents and to within a few tenths of 1% over limited ranges. This instrument is essentially a relaxation oscillator, the frequency of which is proportional to the input voltage. Franklin and Roth (*46*) have described a "colorimetric" chemical coulometer in which the amount of some suitable colored substance produced or consumed at an electrode is measured by the change in absorbancy of the solution. By employing acid-base indicators and electrode reactions which resulted in a change in the pH of the solution, quantities of electricity as small as 0.01 coulomb could be measured accurately. It would appear that coulometers of this type could be devised for measuring coulombs over the entire range normally encountered in coulometric titrations. By using a recording spectrophotometer it should be possible to obtain an automatic recording of coulombs as a function of time. Other instrumental methods, such as polarography (*33*) may be used instead of absorptiometry for determining the changes in concentration. The "coulometric" coulometer of Ehlers and Sease, which was mentioned in the previous review, has since been described in detail (*40*). Yamada and Kondo (*160*) have described a microcoulometer consisting of a capillary tube containing two threads of mercury separated by a small drop of electrolyte. When current is passed between the two mercury electrodes the drop migrates, and the distance of migration is proportional to the number of coulombs passing through the cell.

An improved version of the DeFord (*31*) two-arm cell for the external generation of acids and bases has been described by Bett and coworkers (*8*). The improved cell may be used with generation currents up to 1 ampere. A new type of single-arm external generation cell has been employed by Fuchs and Quadt (*47*) for the generation of acids and bases as well as of iodine. This cell consists of a working electrode in a porous tube, through which the generator electrolyte flows, and which is surrounded by static electrolyte in which the auxiliary electrode is placed. Ion exchange resins placed in the static electrolyte absorb products of electrolysis formed at the auxiliary electrode and prevent their diffusion into the working electrode chamber. Improved cell designs for coulometric analyses involving reductions at a mercury cathode have been presented by Meites (*100*).

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Amperometric Titrations

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THE scope of the amperometric titration method has widened considerably since the last review (*114*) by extension of the types of indicator electrode reactions as well as the types of titration reactions. Application to organic determinations has been particularly active.

This review covers approximately the period July 1, 1951 to October 1, 1955. During this period, several investigators have contributed greatly to the unification of the theory of various electrometric titration procedures (*2, 16, 17, 28, 29, 40, 48–51, 73, 94, 99, 153, 176*). In particular, Kolthoff (*99*) has classified the various methods based on measurement of current according to whether one or two indicator electrodes are used and has thereby classified the dead-stop method as an amperometric method using two identical indicator electrodes. He has concluded that amperometric methods generally have advantages regarding speed, simplicity, accuracy, and sensitivity over potentiometric titrations at zero current or at constant current and that the amperometric method using one indicator electrode is usually superior to the two-electrode systems.

The topic has been reviewed in several articles on electroanalytical methods (*6, 30, 107, 182, 196*) as well as in more specific articles on amperometric titrations (*80, 96, 100, 110, 114, 136, 187*).

The scope of the present review has been extended to include methods such as the dead-stop end point and others closely related to the amperometric method. On the other hand, the field of coulometric determinations has expanded so much that only those are included in which an amperometric end point indication was used.

APPARATUS AND METHODOLOGY

A cell for titrations with the dropping mercury electrode has been described by Gordon and Urner (*53*). A simple apparatus requiring no external amplified e.m.f. has been devised by Ishibashi and Fujinaga (*67*).

A unitized assembly for the rotating platinum electrode was constructed by Agazzi and others (*4*). Other apparatus involving similar electrodes have been described by Herbert and Denson (*60*), Grimes and others (*55*), Konopik (*111*), Mader and Frediani (*124*), and Seagers and Frediani (*163*). Apparatus involving a vibrating electrode has been used by Lindsey (*122*) and Harris and Lindsey (*56*). Apparatus suitable for dead-stop titrations was devised by Dubois, Maroni, and Walisch (*38*) and by van Pelt and Keuker (*141*). Circuits designed to give warning of the approach of a dead-stop end point have been described by Collier and Fricker (*25*) and by Glastonbury (*52*). Automatic titrations have been described by Juliard and van Cakenberghe (*73*) and Stock (*174*). An apparatus for automatic coulometric titrations involving amperometric end points was devised by Richter (*154*).

Kolthoff and Jordan have investigated "convection electrodes" of platinum (*104*) and mercury (*105*) which should have exceptionally favorable characteristics (rapid attainment of steady state, efficient stirring, high sensitivity, reproducibility) for certain types of amperometric titrations.

ION COMBINATION REACTIONS

In the titration of chloride with silver, the necessity of extrapolation from a sufficient distance beyond the end point to eliminate error due to solubility has been demonstrated by Kolthoff and Kuroda (*106*). Varieties of dead-stop end points have been applied to the determination of chloride by Deschamps (*34*) and by Samson (*159*), of iodide by Michalski and Zuk-Kunaszewska (*129*), of halides, cyanide, and thiocyanate by Kies (*95*), and of iodide, bromide, and chloride in successive titrations by Masten and Stone (*128*).

The determination of silver by iodide titration has been described by Parks and Lykken (*138*) using an ammoniacal medium. Dolezal, Hencl, and Simon (*37*) used ethylenediaminetetraacetic acid (EDTA) as a complexing agent to prevent the interference of various heavy metals. A similar titration of thallous ion with iodide in the presence of ethylenediaminetetraacetic acid was described by Přibil and Zabransky (*152*). Songina (*171*) used 0.1*M* iodide as a precipitating reagent for silver and thallium. Songina, Voĭloshnikova, and Kozlovskiĭ (*173*) described the titration of silver in the presence of copper and iron. An amperometric end point was used by Kowalkowski, Kennedy, and Farrington (*112*) for the coulometric titration of iodide with electrolytically generated silver ion.

The precipitation of copper(I) with thiocyanate was described by Liberti (*119*). The diffusion current of copper at the dropping mercury electrode at −0.3 volt was observed.

The precipitation of ferricyanide with silver ion, yielding silver ferricyanide was studied by Khosla and Gaur (*90*). Potassium ferricyanide was used as a reagent for silver, copper, cobalt, nickel, zinc, and cadmium in aqueous or aqueous ethyl alcohol solutions (*75*). Ferricyanide has been used for the determination of zinc in brass and zinc plating baths (*147*). The precipitation of lead (*92, 93*) and zinc (*41, 91, 139, 171, 193*) with ferrocyanide has been investigated. Generally, the diffusion current of the lead or zinc is measured, although Songina (*171, 172*) and Parks, Smith, and Radding (*139*) have observed the anodic current of ferrocyanide at the rotating platinum electrode.

The precipitation of barium chromate has been used for the determination of barium by Vasil'ev and Popel (*185*) and for the indirect determination of sulfate by Warshowsky, Shook, and Schantz (*186*). Ballzco and Schenk (*5*) determined barium in the presence of strontium by collecting the barium selectively on an alumina column. The barium was eluted from the column and precipitated as the chromate, and the excess chromate determined by amperometric titration with standard ferrous sulfate.

The titration of lead with dichromate was used by Hibbits and Cooper (*61*) in the determination of the solubility of lead thiocyanate. The same titration was adapted to the determination of organic sulfur by Rulfs and Mackela (*157*), using a Carius sealed tube digestion in the presence of lead nitrate to form lead sulfate which was dissolved in an acetate buffer and titrated with dichromate. Lead has been used as a precipitating reagent for chromate (*68*), sulfite (*53*), sulfate (*53, 66, 68, 167*), oxalate (*68*), vanadate (*89*), and silicate (*8*). In each case the diffusion current of lead at the dropping electrode was observed.

An amperometric determination of potassium by addition of an excess of sodium tetraphenylboron, and titration of the excess with thallous nitrate in the presence of sodium nitrate as supporting electrolyte, was described by Kemula and Kornacki (*86*). Applications of the older dipicrylamine method are mentioned in the section on organic reagents.

Phosphate has been titrated with iron(III) by Hubicki, Wiacek, and Wysocka (*65*) using a platinum electrode and by Saikina and Toropova (*158*) using a dropping mercury electrode. Arsenate was titrated in a similar way (*157*). Uranyl acetate was used as a reagent by Cogbill, White, and Susano (*24*). Precipitates of the type $MeNH_4PO_4$ have been utilized in the indirect determination of zinc, magnesium, cadmium, and phosphate (*170*). The precipitate was dissolved in excess EDTA, the excess EDTA was removed by addition of ferric nitrate, and the ammonia titrated with hypochlorite in the presence of bromide, using the rotating platinum electrode. Pyrophosphate and triphosphate have been titrated with trisethylenediaminecobalt(III) and with hexamminecobalt(III) ion using the developing mercury electrode (*123*).

Thorium has been determined by fluoride titration using ferric perchlorate as an amperometric indicator (*179*). Gordon and Stine (*54*) titrated thorium in the presence of rare earths using ammonium molybdate as the reagent. The mole ratio of molybdenum to thorium varied somewhat with the amount of thorium taken. Kamecki and Suski (*84*) described a titration of copper(II) sulfate with sodium carbonate using either one or two copper indicator electrodes.

The dead-stop end point has been applied by Dubois, Maroni, and Walisch (*38*) to the titration of acids and bases. Kamecki and Slabon (*83*) titrated solutions of copper sulfate, ferric nitrate, and zinc chloride with sodium hydroxide, using the rotating platinum electrode. An acid-base titration has been used by Kemula and Siekierski (*87*) as an indirect method for the determination of oxygen. Oxygen is reduced by mercury to hydroxyl ion, which is titrated with hydrochloric acid or an acetate buffer at an applied e.m.f. of −1.8 volts.

Several titrations based on complex formation have been described. Dobrowolski (*36*) and Songina (*171*) performed titrations of mercury(II) with iodide using platinum indicator electrodes. Nickel and copper were titrated with cyanide in an ammoniacal solution by Ohashi (*132*). Usatenko and Bekleshova (*184*) determined zirconium(IV) as hexafluorozirconate by titration with fluoride ion, using iron(III) as an amperometric indicator. Aluminum and boron interfered, but silica, calcium, magnesium, titanium, chromium, and iron(II) did not. Kadic and Rezak (*74*) titrated fluoride with ferric chloride in 50% ethyl alcohol containing excess sodium chloride.

Castor and Saylor (*23*) used the aluminum(III) complex of the dyestuff Superchrome Garnet Y (sodium salt of 5-sulfo-2-hydroxybenzeneazoresorcinol) as an amperometric indicator for the determination of fluoride. A decrease in diffusion current of the aluminum(III)-dye complex and a corresponding increase in the diffusion current of the free dye occur in the presence of fluoride. Pecsok and Juvet (*140*) used an amperometric titration of sodium gluconate with copper(II) to study the formation of copper(II)-gluconate complexes.

Ethylenediaminetetraacetic acid has been applied in several titrations. Přibil and Matyska (*149*) described the titration of bismuth, iron(III), lead, cadmium, and nickel. Detailed procedures were devised for bismuth in the presence of many other metals. Pickles and Washbrook (*146*) titrated zinc with EDTA, and Wise and Schmidt (*192*) recently used the reverse titration to determine free EDTA in the presence of the calcium complex. Interference from the magnesium complex was prevented by the addition of glycine. The titration of copper(II) with EDTA was used by Ackermann and Schwarzenbach (*1*) for a study of the replacement reaction between copper(II) and the cadmium-EDTA complex. Přibil and Roubal (*150*) observed that in 4 to 5*M* ammonia, the zinc-EDTA complex is decomposed by calcium ion, and Přibil and Vicenova (*151*) devised a method for the determination of calcium in the presence of barium or magnesium on this behavior. Enough zinc was added to the strongly ammoniacal calcium solution to give a 1 to 2m*M* solution of zinc, which served as an amperometric indicator. At an applied e.m.f. of −1.5 volts the calcium was titrated with EDTA, and the end point was observed as the beginning of decrease of the zinc wave. A method similar in principle, but based on the zincate ion rather than the tetrammine zinc ion, was independently developed by Laitinen and Sympson (*115*), who used 0.20 to 0.28*M* potassium hydroxide as the medium. Michel (*130*) used the anodic depolarization wave of EDTA at a dropping electrode in buffered solutions of pH 6.4 to detect excess EDTA, and found that manganese, cobalt, nickel, cadmium, mercury(II), and zinc gave normal amperometric curves. On the other hand, the alkaline earth cations gave abnormal curves, because the rate of dissociation of the relatively unstable complexes at the electrode surface was great enough to yield an appreciable kinetic current.

OXIDATION-REDUCTION REACTIONS

Methods Based on Iron. The titration of dichromate with iron(II) has been applied to the indirect determination of barium through the precipitation of barium chromate by Ballczo and Schenk (*5*).

Boszai and Hegedus (*14*) described an indirect determination of tellurium through oxidation to tellurium(VI), reduction with calcium hypophosphite, treatment with excess dichromate, and titration with iron. The same authors (*13*) determined manganese by oxidation with peroxydisulfate and titration with iron(II). Parks and Lykken (*137*) determined the copper and iron content of oils by reduction in a silver reductor, receiving the copper(I) in iron(III) solution, and titration of iron(II) with dichromate. Iron alone was determined on a separate aliquot in which the copper(I) was selectively air-oxidized prior to titration. Iron(II) perchlorate was used by Hinsvark and Stone (*62*) as a standard reductant in glacial acetic acid. Using a dead-stop method, solutions of sodium permanganate and chromium(VI) oxide were successfully titrated. The enhanced reducing properties of iron(II) in the presence of EDTA have been used by Přibil, Dolezal, and Simon (*148*) for a reductometric determination of silver. The titration of titanium(III) in the presence of complexing agents, using iron(III) as the oxidant, has been applied to the determination of titanium in steel (*143*).

Furman, Bricker, and Dilts (*45*) used an amperometric end point in the coulometric determination of uranium, based on addition of excess iron(III) to a solution of uranium(IV) and determination of the resulting iron(II) by electrolytically generated cerium(IV). A similar determination of ferrocyanide with electrolytically generated cerium(IV) was described by Dilts and Furman (*35*). Furman, Reilley, and Cooke (*46*) described the coulometric determination of microgram quantities of vanadium in uranium, based on the reduction of vanadium(V) to vanadium(IV) by electrolytically generated iron(II). A similar method, involving a dead-stop end point using a weight microburet had been described for milligram quantities of vanadium by Gale and Mosher (*47*).

Methods Based on Halogens. Harris and Lindsey, using their vibrating platinum electrode, carried out reactions of iodine with thiosulfate and arsenite (*56*), and titrated arsenic(III) and antimony(III) in acidic medium containing bromide, using potassium bromate as the reagent (*57*). Arsenic has been determined in mineral waters by amperometric titration using the rotating platinum electrode (*98*). Bromate was used by Blazek (*10*) for determination of hydroxylamine using similar conditions.

Bradbury and Hambly (*17*) made an amperometric study of departures from stoichiometry of the iodine-thiosulfate reaction and concluded that blanks with the starch indicator method were six to eight times larger than those with the amperometric method. Knowles and Lowden (*97*) found the amperometric end point superior to the starch, dead-stop, or "derived polarographic" end points. Songina and coworkers (*171*, *173*) described iodometric methods for copper and iron. Tutundzhic and Mladenovic (*183*) described the coulometric determination of thiosulfate by electrolytic generation of iodine and proposed the use of the coulomb as a primary reference standard for volumetric solutions. Rowley and Swift (*155*) used an amperometric detection of excess iodine in coulometric determinations and described indirect determinations of iron(III) and dichromate. The same authors (*156*) described two different methods of determining selenium through the iodine-thiosulfate coulometric reaction. The first involves the addition of excess iodide, addition of excess thiosulfate, and titration by electrolytically generated iodine. The second involves the addition of excess thiosulfate, with the formation of selenopentathionate, Se(S$_2$O$_3$)$_2^{--}$, and tetrathionate. The excess thiosulfate is determined as before.

Farrington, Meier, and Swift (*42*) used an amperometric detection of traces of bromine [and copper(I)] as intermediates in coulometric methods involving copper(II) bromide solutions.

Stone (*175*) used a dead-stop end point to follow the reaction of iodide, in the presence of chloroform, with iodate. Bromide caused high results. Peters and Jungnickel (*145*) described improvements in the dead-stop end point for the Karl Fischer water determination.

Iodide was titrated by Kolthoff and Jordan (*103*) to iodine cyanide or iodoacetone using permanganate, cerium(IV), or iodate as the oxidant. Iodate was used by Monand (*131*) as the oxidant for sulfide in the Zimmermann microsulfur determination, involving decomposition of the sample by fusion with potassium. Marks and coworkers (*125–127*) determined residual chlorine in water by titration with phenylarsene oxide. The same reagent was used by Kramer and coworkers (*113*) for the determination of microgram quantities of iodine in water. Various methods for the determination of free chlorine in water have been compared by Whitlock (*189*).

Hypobromite was used by Kolthoff, Stricks, and Morren (*109*) as a reagent for ammonia. Arsenite was used for standardization of the hypobromite. Simon, Sekerka, and Dolezal (*170*) used the titration of ammonia in bicarbonate medium containing bromide, with calcium hypochlorite as the reagent, as the basis of indirect determination of zinc, magnesium, cadmium and phosphate through the precipitation of MeNH$_4$PO$_4$ and dissolution in EDTA. Sekerka and Vorlicek (*164*) determined uranium(VI) by a similar precipitation of the ammonium salt and titration with hypochlorite. Laitinen and Woerner (*116*) titrated arsenite, thiocyanate, and ammonia with hypochlorite in a bicarbonate medium containing bromide.

Miscellaneous Redox Titrations. Blazek (*10*) determined hydroxylamine by titration with titanium(III) in an oxalic acid medium at 50° C., using the dropping mercury electrode as the indicator electrode. Samson and Zschuppe (*160*) titrated oxalic acid with cerium(IV) in 0.8N perchloric acid, using two platinum indicator electrodes. Hubicki and Dabrowska (*64*) determined selenious acid by titration with mercurous nitrate, using the rotating platinum electrode. Simon and Grim (*169*) titrated selenites and selenates with ascorbic acid at 60° C. The titration of sulfamic acid with sodium nitrite was described by Hirozawa and Brasted (*63*). The use of potassium diperiodatocuprate(III) and potassium ditelluratocuprate(III) as oxidimetric reagents were studied by Keyworth and Stone (*88*) using two platinum indicator electrodes. Generally, the complexing anion as well as the copper(III) was reduced. Reproducible results were difficult to obtain.

Hartley and Lingane (*58*) developed a coulometric titration of thallium(I) ion to thallium(III) oxide in strongly alkaline medium using electrolytically generated ferrocyanide ion and a potentiometric or amperometric end point. The precision and accuracy were within 0.2% under favorable conditions.

TITRATIONS INVOLVING ORGANIC REAGENTS

Blackburn (*9*) used 8-quinolinol (oxine) for the determination of bismuth in pharmaceutical preparations. Zan'ko and Panteleeva (*197*) titrated magnesium with oxine in the presence of calcium, iron, and aluminum. Ishibashi and Fujinaga (*66*, *67*) used oxine as a reagent for magnesium, zinc, and copper, and α-benzoinoxime as a reagent for copper.

Tomicek and coworkers (*181*) and Simon (*168*) compared dimethylglyoxime, salicylaldoxime, oxine, and mercaptobenizothiazole as reagents for palladium(II). Dimethylglyoxme proved to be the most sensitive, and mercaptobenzothiazole was the most selective reagent. Mercaptobenzothiazole was used by Liberti (*119*) as a reagent for copper.

Peshkova and Gallai (*143*, *144*) compared dimethylglyoxime and cyclohexanedionedioxime (nioxime) for nickel, and found nioxime superior in selectivity. Furildioxime was found unsuitable because of slow coagulation of the precipitate. Ishibashi, Fujinaga, and Kawamura (*69*), observed satisfactory results in both forward and reverse titrations of nickel and nioxime. Nioxime was found suitable by Calzolari and Furlani (*20*) for the determination of cobalt and nickel together but individually.

Diethyldithiocarbamate has been used as a reagent for copper by Okac and Brejskova (*133*) and for copper, cadmium, and zinc by Calzolari (*18*).

Olson and Elving (*135*) titrated zirconium(IV) with cupferron in $2M$ sulfuric acid. Fluoride in 30- to 35-fold excess did not interfere; a higher concentration could be tolerated if aluminum(III) was added. Cupferron was used by Peshkova and Gallai (*142*) as a reagent for titanium. Good results were reported in $1N$ sulfuric acid in the presence of aluminum, nickel, zinc, and chromium.

Okac and Gracova (*134*) reported 1-nitroso-2-naphthol as an analytical reagent for the titration of cobalt or nickel. The titration of iron(III) in an acetate buffer containing alcohol, with 1-nitroso-2-naphthol was studied by Wilson and Lovelady (*191*). Lead interfered, but calcium, nickel, zinc, aluminum, titanium(III), and chromium(III) did not.

Sandberg (*161*) and Yasumori (*194*) determined potassium by titration with the sodium salt of dipicrylamine, in a phosphate buffer of pH 12 at 0° C.

Kolthoff and Johnson (*101*) used *m*-nitrophenylarsonic acid as a reagent for uranyl, thorium(IV), zirconium(IV), and tin(IV). The same authors (*102*) described the titration of tin(IV) and mercury(II) with tetraphenylarsonium chloride.

Shinagawa and coworkers titrated bismuth(II) in the presence of iodide with triphenylselenonium chloride (*165*) and with triethyltelluronium chloride (*166*).

Calzolari and Donda (*19*) used 2,3-dimercapto-1-propanol (BAL) as a titrant for cadmium, copper, and mercury.

DETERMINATION OF ORGANIC COMPOUNDS

Sulfhydryl Compounds. Benesch and Benesch (*7*) described the titration of soluble mercapto groups materials such as blood, using the rotating platinum electrode in a solvent composed of at least 80% ethyl alcohol. Iwaki (*70*) modified this procedure, using an aqueous solution. Weissman, Schoenbach, and Armstead (*188*) reported on the determination of sulfhydryl groups in serums. Hata and Matsushita (*59*) followed the release of sulfhydryl groups in ovalbumin denatured with sodium hydroxide or urea. Talafant and Sevela (*180*) followed changes in the activity of phosphorylase by amperometric sulfhydryl determination. Grimes and coworkers (*55*) determined mercaptan sulfur in hydrocarbons by silver titration. Zweig and Block (*199*) used amperometric titrations to study the effect of heat treatment on sulfhydryl groups in milk. Leisey (*118*) carried out coulometric titrations with electrolytically generated silver ion, detecting the excess silver amperometrically.

Carter (*22*) carried out sulfitolysis of protein S-S groups, obtaining from one mole of disulfide one mole of sulfhydryl, which was titrated with silver nitrate.

Mercaptobenzothiazole was titrated with silver ion by Liberti (*119*) and with tetramminecadmium(II) ion by Yoshida and Kurihara (*195*). Cadmium ion was used by Calzolari (*19*) for the determination of 2,3-dimercapto-1-propanol.

Larson and Jenness (*117*) used iodine or o-iodobenzoate as a reagent for protein sulfhydryl determinations.

Liberti and Cervone (*120*) titrated thioglycolic acid with mercury(II). Kolthoff, Stricks, and Morren (*108*) and Stricks, Kolthoff, and Tanaka (*178*) described the use of mercury(II) as a titrant for various types of sulfhydryl groups. Stricks and Kolthoff (*177*) determined oxidized glutathione in the presence of cystine. The determination was based on the hydrolytic fission of oxidized glutathione

$$2\,\mathrm{GSSG} + 3\mathrm{OH}^- \rightarrow 3\mathrm{GS}^- + \mathrm{GSOOH} + \mathrm{H_2O}$$

followed by amperometric determination of GS^- by titration with mercuric chloride using the rotating platinum electrode.

Halogen Titrations. Braae (*15*) applied the dead-stop end point to the reaction of olefins with bromine, and Blazek (*11*), using the rotating platinum electrode determined the iodine numbers of 25 unsaturated compounds by titration with bromine in glacial acetic acid. Duke and Maselli (*39*) used an amperometric end point to determine iodine values according to Hanus, Benham-Klee, and Wijs for several fatty acids and oils. Liberti and Cervone (*121*) titrated thiourea with iodine.

Carson (*21*) used electrolytically generated bromine for the coulometric determination of 8-quinolinol (oxine). Kawamura, Momoki, and Suzuki (*85*) determined salicylic acid by adding an excess of electrolytically generated bromine and determining the excess by reversing the polarity and back-titrating with electrolytically generated copper(I), using an amperometric end point. Cuta and Kucera (*31*) used electrolytically generated chlorine to determine long-chain unsaturated fatty acids.

Potassium iodate has been used as a titrant for ascorbic acid by Coulson, Crowell, and Friess (*27*), who avoided the interference of glutathione by treatment with an excess of 2,6-dichlorophenolindolphenol and determination by difference.

Potassium bromate was used by Duyckaerts and Pitance (*41*) as a titrant for oxine and by Fernando (*43*) for oxine and some of its derivatives. Liberti (*119*) titrated *p*-aminosalicyclic acid with potassium bromate.

Other Organic Determinations. A dead-stop titration of amines by diazotization was described by Ferrero and Brehain (*44*), who titrated primary aromatic amines with standard sodium nitrite in the presence of hydrochloric acid, and by Scholten and Stone (*162*).

Adams, Reilley, and Furman (*3*) carried out a "derivative polarographic titration" of glucose with potassium ferricyanide at the boiling point, with the pH controlled by sodium carbonate.

Bogdanov and Sukhobokova (*12*) used cerium(IV) sulfate in $2N$ sulfuric acid, with the rotating platinum electrode as an indicator electrode, to titrate several organic compounds, including hydroquinone, pyrocatechol, *p*-aminophenol, metol, and *p*-phenylenediamine.

Silicotungstic acid was used by DeAngelis (*32*) as a reagent for nicotine and by Willits and Ricciuti (*190*) for nornicotine. In order to obtain a stable precipitate of definite composition, the reagent had to be added at 15-minute intervals.

Zuman and Prochazka (*198*) titrated ascorbic acid with 2,6-dichlorophenolindophenol dye.

Kalvoda and Zyka (*79*) used lead nitrate as a precipitant for glycerolphosphates, tartaric acid, and tartrates, and carried out extensive investigations of the use of mercury(II) salts as titrants for various pharmaceuticals (*76–79, 81, 82*) such as salicylates and barbiturates. Jindra, Jungr, and Zyka (*71*) and Jindra, Kalvoda, and Zyka (*72*) used *p*-diazobenzenesulfonic acid as a titrant for various alkaloids.

Conn and Norman (*26*) based an amperometric determination of streptomycin and dihydrostreptomycin on their quantitative precipitation with various anionic dyes in suitable buffer solutions.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Potentiometric Titrations

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THIS paper attempts to summarize the chief trends in the development and application of potentiometric titrations since the time of the prior review in the series by Furman (*50*).

Elving (*44*) has reviewed the current status of analysis by titration and summarized several trends in development and application with respect to reactions, titrants, measurement of titrants, media for titration, and detection of the equivalence point. Langford (*89*) covers various methods applicable in the electroplating industry. Codell, Norwitz, and Mikula (*28*) have reviewed the new methods of titanium alloy analysis. The methods for the determination of free chlorine have been surveyed by Whitlock (*174*). West (*173*) has reviewed the topic of titrations in anhydrous solvents. Waters, Berg, and Lachman (*172*) have surveyed the scope of potentiometric titrations in pharmaceutical control.

THEORETICAL DEVELOPMENTS

Interpretation of Polarization Curves. On the basis of Nernstian equilibria and kinetics, Jordan (*71*) interpreted the reversibility and irreversibility of simple electrode reactions in terms of two competing rate processes. Jordan derived a generalized wave equation for the current-voltage curves obtained in "hydrodynamic voltammetry" for the case where the competing rate processes are mass transfer and electron transfer:

$$i = \frac{i_l}{\dfrac{m_{\text{ox}}}{k_{\text{ox}}\, f_{\text{red}}} + \dfrac{m_{\text{ox}}\, f_{\text{red}}\, K}{m_{\text{red}}\, f_{\text{ox}}} + 1}$$

where i_l is the limiting current (amperes), m denotes mass transport coefficients (centimeters per second) for the oxidized and reduced species, f denotes the activity coefficients, and K corresponds to the ratio (k_{ox}/k_{red}) of the first-order rate constants (centimeters per second) for the forward and reverse step of the reaction

$$\text{Red} \underset{k_{ox}}{\overset{k_{red}}{\rightleftarrows}} \text{OX} + ne$$

The prediction and interpretation of the form of the curves obtained in bimetallic potentiometric titrations have been pointed out by Coursier (*31*) through the use of polarization curves. Four cases are distinguished: (1) Both the oxidized and reduced forms of the two systems are electroactive at the two electrodes, (2) both systems have only one electroactive form, (3) the titrant has only one electroactive form, and (4) the system titrated has only one electroactive form.

Kies (*79*) derived a theoretical equation for the intensity of current as a function of added reagent during a dead-stop titration for reversible systems.

Polarization curves were used by Coursier (*30*) to explain certain effects which occur in the neighborhood of the equivalence point in a potentiometric titration, such as slow attainment of an equilibrium potential. Duyckaerts (*42*) has presented an excellent summary of recent developments in the interpretation of electrotitrimetric methods in terms of polarization curves.

Kolthoff (*81*) has reviewed the relationships among voltammetry, potentiometry, and amperometry and has presented equations and data to illustrate these relationships quantitatively.

Charlot (*25*) has also pointed out the significance of polarization curves. Piontelli and others (*115*) have described exact methods for measuring polarization voltages.

Adams (*2*) extended the technique of controlled current-scanning polarography to electrometric titrations. The dropping mercury electrode was employed for the titration of cadmium with ethylenediaminetetraacetic acid [(ethylenedinitrilo)tetraacetic acid]. In this method a small constant current, of about 1μa., was applied between the calomel and dropping mercury electrodes. The resulting potential drop across the two electrodes was measured during the course of the titration, with the break occurring just prior to the equivalence point.

Tegze (*155*) has studied titrations in which the end point is indicated by measuring voltage at constant current. Included in his study were argentometric titrations with silver electrodes; neutralization titrations with hydrogen, quinhydrone, and antimony electrodes; oxidation-reduction titrations; and reactions involving complex formation.

A new principle of end-point detection termed "chronopotentiometric" has been described by Reilley and Scribner (*124*). The method is based upon measurement in quiet solutions of the "transition times" for a species during the course of a titration. In operation, a constant current is applied suddenly across an electrode-solution interface. The resulting potential then progresses rapidly to the decomposition potential of the species, remains there until the species at the interface has been depleted entirely, and finally moves to the decomposition potential of some other species or the solvent. The square root of the time (transition) required for complete depletion is directly proportional to the concentration of the species. The titration curves are straight lines essentially analogous to amperometric titration curves. The method is particularly applicable to titrations of small volumes and to situations where stirring is undesirable.

Potential-pH Diagrams. Sillen (*140*) has discussed the usefulness of redox diagrams of which potential-pH diagrams are a special case. These diagrams summarize a large number of competing equilibria into a readily digestible diagram whereby one may obtain quickly a clear picture of how the various equilibria interact. Charlot (*26*) has also discussed application of potential-pH diagrams and polarization curves in analytical chemistry.

Determination and Use of Equilibrium Constants. Vanderbelt, Henrich, and Vandenberg (*164*) have compared the determination of $\mathrm{p}K_a'$ values by electrometric titration and ultraviolet absorption methods. These methods are in good agreement. The titration method is more rapid, especially when two or more ionizable groups are present in a molecule. When the $\mathrm{p}K_a'$ values are close—e.g., adjoining pH units—the absorption method is extremely difficult to apply. The absorption method is not so generally applicable, as appreciable spectrum changes are not always obtained, especially in the case of some carboxyl derivatives. The absorption method is the more desirable for very large or very small $\mathrm{p}K_a'$ determinations where pH or pOH values are near unity. It is also useful in studying materials of low relative solubility.

Apparent dissociation constants determined within ±0.05 $\mathrm{p}K'$ unit by potentiometric titration may be employed in qualitative organic analysis on a milligram basis as described by Parke and Davis (*111*). A chart of $\mathrm{p}K_a'$ values for 573 compounds is given.

The hydrolysis constants of 12 *n*-primary amines in 22% ethyl alcohol were determined by Slabaugh and Cates (*146*) from potentiometric titration data. Kieffer and Rumpf (*78*) determined the dissociation constants and structures of 14 phenolic compounds by potentiometric and spectrophotometric methods.

The formation of ion pairs becomes particularly prominent in media of low dielectric constant, often resulting in inaccurate inflection points in the potentiometric titration of acids and bases in nonaqueous solvents. Troublesome salt effects on color indicators may also be expected. The physical nature of the ion pairs and the magnitude of their dissociation constants have been reviewed by Grunwald (*56*).

Bates (*8*) has investigated the effect of dilution upon the pH of a buffer and has coined a unit, the "dilution value," $\Delta\mathrm{pH}_{1/2}$, the change in pH accompanying dilution of the buffer solution with an equal quantity of water. Two separate effects operate. The first is the effect of the acid-base equilibrium of water upon the buffer equilibria, causing a shift toward a pH of 7 upon dilution. The second is the change in activity coefficients upon dilution. Bates (*9*) has published a comprehensive book covering the electrometric measurement of pH as well as properties and standardization of buffer solutions.

The metal-chelating tendencies for a large number of chelating agents have been measured by the Bjerrum method. Lumb and Martell (*94*) have employed the method in the case of glutamic and aspartic acids.

Kordatzki (*83*) has reviewed the measurement of oxidation-reduction (rH) potentials. A series of "formal" potentials has been reported by Bock and Hermann (*13*) for the iron(III/II) aqueous system at various strengths of perchloric, nitric, hydrobromic, hydrochloric, sulfuric, phosphoric, acetic, and hydrofluoric acids. The potential in 1.0*N* perchloric acid was 0.747 volt *vs.* the normal hydrogen electrode, and descending in the above order to 0.320 volt in hydrofluoric acid.

Sillen (*139*) has reviewed his excellent previous work on the electrometric determination of the equilibria between mercury and halide ions. Studies of electrode potentials in fused potassium chloride–lithium chloride media by Senderoff and Brenner (*135*) showed evidence for the formation of chloride complexes in the case of zinc, lead(II), iron(II), tin(II), and copper(I).

Ricci (*125*) made a theoretical study of the relation of the inflection point of the p[Ag$^+$] curve to the equivalence point and to the first appearance of a precipitate. He also considered the effect of the addition of ammonia.

The Coulomb as a Primary Standard. Utilizing the fact that electrons are a most versatile reagent, Tutundzic (*163*) has proposed that the coulomb (by way of coulometric titrations) be considered as the "primary standard" for all volumetric standardizations.

Statistical Studies. Linnig, Mandel, and Peterson (*93*) have described the statistical methods for use in studying the accuracy and precision of an analytical procedure through segregation into relative-type and constant-type systematic errors. Included is a discussion of the effect of proper choice of indicator based on a pH titration curve.

APPARATUS

Instruments. The general field of automatic potentiometric titrations has been reviewed by Kimoto (*80*). Simon and Heilbronner (*141*) describe an automatic apparatus for microtitration of organic compounds. The rate of addition of titrant is governed by the rate of flow of mercury through a thermostated polarographic capillary and consequently can be made rather independent of the usual "thermometer" effect encountered in syringe delivery systems. With this apparatus a number of organic acids and bases were automatically titrated in methyl Cellosolve–water or dimethylformamide-water media and their dissociation constants determined. Richter (*126*) developed an instrument for performing automatic coulometric titrations using an amperometric end point.

Wight and Burk (*175*) developed a technique for continuous recording of oxidation-reduction potential and pH changes in manometric vessels used in studies on dynamic biological systems. Eades and coworkers (*43*) developed an automatic titrating and recording apparatus for microbiological assays, which can handle 225 samples consecutively and yield determinations on acids and bases within 1% standard error.

An apparatus for fully automatic determination of both ionization constants and enzymatic activity has been described by Neilands and Cannon (*106*). The kinetic analysis was accomplished by following with time the volume of titrant necessary to maintain constant pH conditions in a slowly reacting solution. The hydrolysis rate of triacetin by acetyl esterase of orange peel at pH 7.00 and the reduction rate of diphosphopyridine nucleotide by lactic dehydrogenase of heart at pH 9.50 were illustrative examples.

A potentiometric device has been developed by Ingraham and Makower (*65*) to determine automatically the chronometric end point, which serves as an estimation of polyphenol oxidase activity. The principle is based on the enzymatic reaction in which *o*-quinone is continually produced and reacts in turn with an added amount of ascorbic acid. After a given length of time the ascorbic acid is entirely consumed and the potential change occurring between a platinum–saturated calomel electrode system electromatically shuts off the timing mechanism.

The Beckman automatic titrator was adapted by Jones and Baum (*70*) for the automatic titration of chloride obtained from chlorinated organic compounds. An automatic trigger circuit useful for stopping the addition of titrant at the end point has been developed by Carson (*21*) especially for use in coulometric titrimetry.

Malmstadt and Fett (*97*) have developed an automatic differential potentiometric titrator which has special advantages for titrations where the end-point potential is not known prior to titration or where the end-point potential changes rapidly with different titration conditions. Because the automatic differential titrator is not suited for titrations where the solution or electrodes reach equilibria very slowly, Malmstadt and Fett (*98*) investigated the potential response characteristics of various indicator electrodes. For acid-base titrations they studied glass, antimony, platinum, platinum–10% rhodium, platinum–40% rhodium, and graphite indicator electrodes, with the platinum–10% rhodium electrode exhibiting the most rapid response. This latter electrode also could be used advantageously in nonaqueous solvents—benzene-methanol or glacial acetic acid. Antimony electrodes were sluggish in their response but, with appropriate corrections, good results were obtained. Hydrazine was titrated with standard iodate by the Andrews method using platinum-calomel. No lag in the electrode response was detected. Malmstadt and Fett also titrated successfully chloride with silver nitrate and with mercuric nitrate. Cyanide was titrated with silver nitrate with no time delay in electrode response.

Harwell (*60*) developed a stable electronic voltmeter for use in the accurate determination of the "chlorinity" of sea water by titration with silver nitrate.

A simplified dead-stop, magic-eye, end-point indicator has been developed by McCauley and Gresham (*95*) for routine Karl Fischer titrations.

A zero grid current vacuum tube voltmeter has been applied by Natelson (*105*) to the precise (±0.002 pH unit) measurement of pH with the glass electrode. Hallikainen and Pompeo (*59*) have devised a continuous electrometric titrator.

Tubbs (*162*) has described the use of two transparent templates, inscribed with concentric arcs, for rapid location of the inflection point of potentiometric titration curves.

Parsons, Seaman, and Amick (*112*) employed a small low-inertia motor for integrating the current-time relationship in coulometric titrations, thus avoiding the necessity for using regulated current supplies. They employed electrolytically generated ferrous ion, silver ion, and hydroxide ion for the titration of dichromate, chloride, and hydrochloric acid, respectively.

Gerhardt, Lawrence, and Parsons (*51*) describe a precision coulometric titrator capable of carrying out coulometric titrations within 0.1% precision and accuracy, supplying constant currents up to 450 ma.

Electrodes. A comprehensive study of residual current-voltage phenomena of platinum electrodes resulting from sorbed hydrogen and formation and dissolution of platinous oxide films has been made by Kolthoff and Tanaka (*82*). Chemical pretreatments for minimizing these effects are described.

Purdy, Burns, and Rogers (*119*) investigated in detail the sensitivity of the bromine-bromide potentiometric end point with particular regard to the factors contributing to the reagent blank in a coulometric titration.

Janz and Taniguchi (*69*) have written a very comprehensive review on the preparation, stability, reproducibility, and standard potentials in aqueous and nonaqueous media of the silver–silver halide electrodes. Gordon (*54*) describes a sturdy, easily resurfaced silver indicator electrode useful in halide determinations.

Achiwa (*1*) suggested the use of metal–metal sulfide indicator electrodes in neutral titration of metal ions.

Hubbard (*63*) has studied the temperature effect on electrical resistance and voltage errors of glass electrodes. Glass electrode failures were attributed to low hygroscopicity of the glass, excessive wall thickness, poor chemical durability, high electrolyte concentration, and inhibiting films. Heyn and Bergin (*61*) attempted without success to make a glass electrode which would function in liquid ammonia systems.

Carson and Michelson (*22*) investigated the effect of radioactivity on the pH response of glass electrodes. No effect was observed after exposure to 200 curies per gallon of β-activity, 300,000 r. per hour of γ-radiation, or 0.5 mc. per gallon of α-activity.

Mantzell (*99*) has investigated the effect of electrical polarization on the precision of pH measurements made with the antimony electrode. Rabinovich and Gorbunova (*120*) investigated the potential and temperature coefficient of the antimony electrode in the intervals pH 2 to 12 and 20° to 50° C. An effect caused by citric acid or boric acid was noted.

Rabinovich and Kurovskaya (*121*) described the preparation of a rugged platinum electrode made simply by metallizing the glass in a flame with a special mixture containing chloroplatinic acid, rosin, alcohol, and boric acid.

A metallic mercury electrode covered with solid mercurous acetate was found by Scarano and Ceglie (*129*) to be useful as an indicator electrode for neutralization reactions in glacial acetic acid solvents.

Sinha (*144*) discusses the possible application of ion exchange resin membrane electrodes for pH measurements in the absence of other cations.

Fuji and Ishikawa (*49*) have investigated the preparation and use of palladium hydride electrodes for pH measurement. A mean error of only 0.001 pH unit is claimed in simple systems. Kunze (*86*) has proposed the use of a chromic chromate, $Cr_2(OH)_4CrO_4$, electrode as an indicator electrode, especially in alkaline solutions where the quinhydrone electrode is unsatisfactory.

A paper by Ohlweiler and Meditsch (*107*) describes the general usefulness of the glass electrode as a reference electrode in oxidation-reduction titrations.

Porous glass and ion exchange membranes were adapted by Carson, Michelson, and Koyama (*23*) for use in salt bridges to obtain greater mechanical stability, smaller solution flow, and less electrical resistance. Brunisholz (*17*) has described a salt bridge and reference electrode of particular use in potentiometric titrations.

Lamoen and Borsten (*88*) described the use of a platinized platinum indicator electrode and a shielded bright-platinum reference electrode for potentiometric titrations with Karl Fischer reagents.

APPLICATIONS

Analysis Based upon Measured Potentials. An interesting use of potential measurements was described by Onaka (*108*). By employing the cell

$$\text{Ag/(molten steel)//electrolyte, } H_2\text{/Ag}$$

the potential difference was measured and correlated directly with the oxygen content of the molten steel. An oxygen content of 0.065%, for example, corresponded to a potential of 160 mv.

Kahlweit, Strehlow, and Hocking (*72*) found that an electrolytic cell of the type

$$\text{Electrolyte } a \text{ in } H_2O \mid \text{electrolyte } b \text{ in oil} \mid \text{electrolyte } a \text{ in } H_2O$$

exhibits an electromotive force indicative of ion exchange behavior. With electrolyte *a* as different concentrations of potassium chloride, and electrolyte *b* as the potassium salt of phenyl quinoline carbonic acid (in quinoline), the system behaved as a cation exchanger.

Solvolytic rate constants were obtained by Kwart and Wilson (*87*) with an apparatus which automatically followed by potentiometric means the course of the reaction. Marshall (*100*) determined the solubility of uranium trioxide in aqueous sulfuric acid at elevated temperatures, 150° to 290° C., by sampling the equilibrated solution at the desired temperature, cooling to 25° C., and comparing the pH of the sample with control pH data. The precision pH measurements were made within ±0.004 pH unit, using a vibrating reed electrometer.

The chloride ion content in a solution was determined by Chanin (*24*) by measuring the potential of a silver-silver chloride electrode immersed in the solution and comparing the measurement with a calibration curve.

Carbon dioxide in air was estimated by Kauko and Icel (*76*) using a concentration cell containing quinhydrone. Through one half cell the gas to be analyzed was bubbled. Pure carbon dioxide was bubbled through the other. By running the gas over iodine pentoxide or Hopcalite II, carbon monoxide could also be determined.

Cuta and Burianac (*34*) followed the concentration of traces (0.003 to 0.5 mg. per liter) of hydrogen sulfide in hydrogen by passing the gas mixture into an absorption device containing a very dilute iodine solution. The content of hydrogen sulfide was determined from the difference in electrode potentials before and after absorption by means of a calibration curve.

Frisque and Meloche (*46*) have described a mathematical method for determining the concentrations of two weak monobasic acids in a mixture in the case where the pK's of the acids are so close together that only one break is observed in the pH titration curve.

An antimony microelectrode was used by Kamienski, Bylo, and Waligora (*73*) for detecting 10^{-2} to $10^{-5}M$ quantities of various acids and bases as they were eluted from an alumina column. The logarithmic relationship between the electrode potential and concentration allowed easy detection of minute quantities. The antimony electrode was also found by Kamienski, Puchalka, and Dolinski (*74*) to be useful in following the elution of stearic and oleic acids from silica gel columns by a benzene-alcohol eluent.

Acid-Base Processes. AQUEOUS. Van Wazer, Griffith, and McCullough (*165*) have described a pH titration procedure for the determination of orthophosphates, polyphosphates, and total phosphorus pentoxide. Titration with base consumes two hydrogens from orthophosphate and end-group hydrogens on polyphosphates. Addition of silver nitrate frees the third proton of orthophosphate to allow determination of orthophosphate. Titration, after hydrolysis in hydrochloric acid to convert polyphosphates to orthophosphates, allows determination of total phosphorus pentoxide.

A rapid volumetric method for mercury proposed by Palit and Somayajulu (*110*) consists of conversion to neutral mercuric oxide, addition of excess potassium iodide or sodium thiosulfate, and subsequent acidimetric titration of liberated alkali. They also recommend mercury salts as primary acidimetric standards. Hall and Stone (*58*) have proposed the use of 4-aminopyridine as a standard in acidimetry. It is easily purified by recrystallization from toluene or benzene and is a moderately weak base (dissociation constant $= 1.3 \times 10^{-5}$).

Larson and Henley (*90*) determined with extreme sensitivity low alkalinity or acidity in rain water. Extrapolation to 1×10^{-7} mole (H^+) per liter was necessary to obtain a precise equivalence point.

Warner and Raptis (*171*) determined formic acid in the presence of acetic and other organic acids by first separating the formic acid by azeotropic distillation with chloroform and subsequently titrating recovered formic acid directly with standard base.

Day and others (*35*) passed an aqueous solution of uranyl sulfate, nitrate, chloride, perchlorate, and dichromate through a cation exchange resin (on hydrogen cycle) and titrated the acid effluent with standard base to determine total anion concentration. Zall, Wagman, and Ingber (*179*) proposed a rapid volumetric acid-base titration for analysis of boiler compounds consisting of sodium carbonate, disodium phosphate, and starch. Smelik and Habiger (*147*) report the application of the antimony indicator electrode for the titration of dark liquors in the sulfate pulping industry.

NONAQUEOUS. Brockmann and Meyer (*16*) developed a micromethod for determining the equivalent and molecular weight of weak organic acids and bases by means of potentiometric titrations in nonaqueous media.

Fritz (*47*) has outlined the general requirements of solvents and titrants for weak acids or bases. The most generally useful solvents for the titration of weak acids are dimethylformamide, ethylenediamine, butylamine, and alcohol-hydrocarbon mixtures. For weak bases acetonitrile or chloroform allows differential titration. Wollish, Pifer, and Schmall (*178*) have pointed to the applicability of titration in nonaqueous solvents for the analysis of pharmaceuticals.

The glass-calomel electrode system has been successfully used by Deal and Wyld (*36*) with alcoholic hydroxide titrants for the estimation of very weak acids, employing ethylenediamine and dimethylformamide alternatively as solvents to enhance the titration response desired. Wollish and others (*177*) have determined each component separately in APC (aspirin, acetophenetidine, caffeine) tablets, employing titration in nonaqueous media in the aspirin and acetophenetidine determinations.

The neutralization equivalent of substituted fatty acids was carried out by Radell and Donahue (*122*) in a benzene-methanol solvent using sodium methoxide titrant and antimony-calomel electrodes. Lithium chloride was added to the solvent to decrease the solution resistance.

Anhydrous pyridine (*157*) served as the solvent for titrations of perchloric acid, formic acid, benzoic acid, and the ion exchange resin, Zeo-Karb. The titrant was piperidine, ammonia, or diethanolamine dissolved in pyridine. A glass electrode was satisfactory for determining the end point.

Glenn and Olleman (*52*) have adapted several known procedures for application to composite analysis of several types of coal hydrogenation products. Carboxylic acids and monohydric phenols were determined by sodium aminoethoxide titration in ethylenediamine, basic nitrogen compounds by perchloric acid titration in glacial acetic acid, primary and secondary amines and alcohols by acetylation with acetic anhydride–pyridine, primary and secondary amines only by acetylation followed by selective hydrolysis of *O*-acetyl groups, and total active hydrogen with Grignard reagent.

The use of the solvent acrylonitrile for the precise determination of small amounts (0.001%) of weak acids and bases has been reported by Owens and Maute (*109*). The method is rapid and free of interference from atmospheric carbon dioxide, and standard glass-calomel electrodes may be used. Glenn and Peake (*53*) employed a glass-platinum electrode system for the titration of phenolic esters in ethylenediamine solvent. Carbon dioxide in ethanolamine was determined by Wagner and Lew (*170*).

Cundiff and Markunas (*33*) replaced the saturated aqueous potassium chloride in a fiber-type calomel electrode with a methanol solution saturated with potassium chloride in the nonaqueous perchloric acid titration used in the determination of nicotine, nornicotine, and total alkaloids in tobacco.

Sulfuric acid yields two sharp breaks when titrated in acetonitrile solvent with morpholine. Critchfield and Johnson (*32*), who investigated this effect, also found that mixtures of sulfuric acid with hydrochloric or nitric acids could be titrated in this manner to determine each component.

Titrations of urea, thiourea, and various substituted guanidines and nitroguanidines were effected by DeVries, Schiff, and Gantz (*37*) using dimethylformamide or trifluoroacetic acid as solvents with antimony-calomel and platinum-platinum electrodes, respectively. Of particular interest is the titration of nitroguanidine and thiourea, materials which are not titratable in glacial acetic acid.

An acetic acid–chloroform solvent was employed by Pifer, Wollish, and Schmall (*114*) for the differential titration of ammonium or potassium acetate (stronger bases) in the presence of other metal acetates. Streuli (*151*) found that basic copolymers of acrylonitrile may be titrated with perchloric acid when a mixture of nitromethane and formic acid is employed as a solvent. Neutralization titrations in anhydrous formic acid using hydrogen or quinhydrone electrodes was employed by Tomicek and Vidner (*160*) for the determination of various organic bases.

Siggia and Stahl (*138*) presented a nonaqueous method for determining aldehydes, which consisted of adding a measured excess of unsymmetrical dimethylhydrazine to the sample, and, after the reaction is complete, back-titrating the excess with standard acid. The solvent employed was ethylene glycol or methanol.

Jackson (*68*) determined primary fatty amines from the difference between the acid required for titration carried out in chloroform in the absence and presence of salicylaldehyde.

Gremillion (*55*) applied the use of acetic anhydride solvent in titrating weak primary and secondary amine bases whose K_b values in water are 10^{-15} or larger. Acetic anhydride was also found useful in titrating the sulfuric acid catalyst in ethyl alcohol esterification mixtures.

Fritz and Fulda (*48*) have investigated the titration of weak bases in acetic anhydride–nitromethane solvent mixtures. Excellent results were obtained with tertiary amines, including salts of purine, pyridine, pyridone, and thiazole types.

Precipitation. Coulometric generation of silver ion was employed in the titration of thiourea and thiocyanate by Nakanishi and Kobayashi (*103*). The coulometric generation of silver ion was also applied by Lingane (*92*) in the determination of chloride or bromide or iodide in amounts ranging from 0.2 to 10 mg. The Beckman automatic titrator was employed to render the method automatic, with titrations completed in 1 to 5 minutes. A similar coulometric method was employed by Kowalkowski, Kennedy, and Farrington (*84*) in the determination of iodide.

Věceřa (*166*) performed microdeterminations of sulfur in organic compounds by combustion on a platinum contact, absorption on silver metal, and subsequent titration of the silver sulfate with iodide.

Perchlorate (*130*) may be determined by reduction to chloride with a titanous, metallic aluminum mix under reflux and subsequent titration of chloride with silver nitrate.

The potentiometric titration of micro quantities of halides (from organic halogen compounds) with silver nitrate, employing a silver–amalgamated silver electrode system, is described by Cogbill and Kirkland (*29*).

A precise determination of chloride in plasma by direct titration with silver nitrate was accomplished by Dole and Thorn (*41*) using a differential potentiometric procedure. The silver–silver chloride electrodes were made in the usual manner by electroplating on platinum electrodes. The presence of bromide, thiocyanate, chlorate, cyanide, and iodide in argentometric determination of chloride was accommodated by oxidation with ceric sulfate and subsequent distillation in the procedure developed by Mahr and Otterbein (*96*). Free cyanide in the presence of sulfides was determined by Karchmer and Walker (*75*) by preliminary potentiometric titration of the sulfide with silver nitrate, followed by polarographic determination of cyanide.

The dead-stop end point was employed by Masten and Stone (*102*) for the successive argentometric titration of a three-component mixture of chloride, bromide, and iodide. Antzel (*6*) has described the electrometric titration of mercury and silver with potassium iodide using a calomel electrode. Only iron(III) interferes. Capitani and Gambelli (*19*) report the selective argentometric titration of cyanamide and dicyanodiamide.

Ikeda and Komooka (*64*) added cadmium carbonate to eliminate interference of sulfide in argentometric titration of chloride. Use of an equal volume of ethyl alcohol minimized the effect of a considerable excess of sulfate ion.

The titration of sulfate, based on addition of excess lead and back-titration with ferrocyanide, has been proposed by Agasyan (*3*). Tananaev, Glushkova, and Seifer (*154*) titrated lanthanum solutions potentiometrically with standard potassium ferrocyanide on the principle of precipitating $KLa[Fe(CN)_6].7H_2O$, which has a solubility of 2.28 to 2.76 $\times 10^{-4}$ mole per liter. A method proposed by Teodorovich and Leushina (*156*) uses the precipitation of copper ions upon addition of potassium ferricyanide to estimate copper.

Vogel (*167*) employed a combined distillation-titration to determine fluoride in the presence of boron, beryllium, titanium, aluminum, or thorium. The fluoride, distilled as H_2SiF_6, is titrated with standard uranyl solutions in the presence of the potassium salt of sulfanilic acid as buffer, utilizing the precipitation of KUF_5.

Sodium ion was titrated in milligram amounts in 85 to 95% aqueous ethyl alcohol by Tomicek and Pulpan (*158*), using uranyl zinc acetate titrant and an indicator electrode of Jena G 20 glass. Potassium in smaller concentrations than sodium did not interfere.

Wittman (*176*) determined ferrous iron by precipitation with ferricyanide.

Complexation. The use of chelating agents, such as ethylenediaminetetraacetic acid, as reagents in titrimetric analysis promises to be one of the most profitable new developments in analysis. Such agents lead to sharp end-point breaks because they combine with the metal ions in a 1 to 1 ratio, thereby eliminating the undesirable formation of lower complexes prior to the end point and the dissociation of higher complexes near the end point. The chelating effect also enhances the stability of the complex and causes a larger break in the end point. Martell and Chaberek (*101*) have described these influences and discussed several methods for detecting the equivalence point in these titrations.

An excellent introduction to the scientific thinking which led eventually to the development of ethylenediaminetetraacetic acid is the recent lecture to the Society for Analytical Chemistry by Schwarzenbach (*132*). Schwarzenbach (*133*) pioneered the development and application of such reagents and has recently collected the pertinent current literature into book form.

Blaedel and Knight (*12*) have described the purification and properties of a primary standard grade of ethylenediaminetetraacetic acid. A $0.01M$ solution of the disodium salt was found to change by less than 0.05% in titer after storage in polyethylene bottles for 5 months.

Siggia, Eichlin, and Rheinhart (*137*) developed a potentiometric titration procedure for analyzing chelating agents, metal ions, and metal chelates as well as mixtures of components, with an accuracy of ±1%. Selective titrations were effected by proper choice of electrode (mercury plated on platinum, silver, or bright platinum), solution conditions (pH, buffer type), and titrant. In this manner iron, copper, mercury, zinc, lead, manganese, calcium, magnesium, nickel, and cobalt could be determined alone. By employing metal ions as titrants, ethylenediaminetetraacetic acid, *N,N*-di(β-hydroxyethyl)glycine, or nitrilotriacetic acid—or mixtures of ethylenediaminetetraacetic and nitrilotriacetic acids—could be estimated.

Chilton and Horton (*27*) describe an improved acidimetric determination of fluoride, whereby the titration of a neutral solution of fluoride with aluminum ions exhibits an abrupt decrease of pH at the stoichiometric end point. The method is applicable in the range 0.1 to 3.5 mg. of fluoride per ml. of water and gives a standard deviation of less than 0.2% with a recording pH meter. Sodium fluoride also was titrated with aluminum salts by Talipov and Teodorovich (*153*) using a quinhydrone electrode.

Oxidation-Reduction. FERROUS METHODS. In the determination of chromium, iron, and silica in chromite ores, Zivanovic (*180*) titrated the chromium (after oxidation to dichromate) with standard ferrous and the iron (after reduction to ferrous) with dichromate.

Trivalent and total cobalt can be determined in the presence of excess tungstate, molybdate, and iron by the method of Baker and McCutcheon (*7*), by introducing excess ferrous ion and back-titrating with dichromate. Total cobalt is determined by oxidation of cobalt to cobalt(III) hydroxide with sodium perborate prior to addition of ferrous iron.

Iron(II) perchlorate can be used in glacial acetic acid for the titration of oxidants, chromium trioxide, and sodium permanganate, by the method of Hinsvark and Stone (*62*).

CHROMOUS. Standard chromous chloride was employed by Bottei and Furman (*14*) for determination of anthraquinones, and nitro, nitroso, azo, and acetylenic compounds using platinum-calomel electrodes. Anthraquinone could be determined directly, while the other compounds required an excess of chromous ion with subsequent back-titration with standard ferric alum solution.

TUNGSTEN(V) AS REDUCTANT. Tungsten(V) prepared by electrolytic reduction of tungstate in $10N$ hydrochloric acid is sufficiently stable under proper storage conditions to serve as an effective reducing agent. Tourky, Issa, and Amin (*161*) employed this titrant for the determination of chromium, iron, copper (in hydrochloric acid), and iodate.

URANOUS AS A REDUCTANT. Electrolytically generated uranous ion was employed by Shults, Thomason, and Kelley (*136*) for the automatic coulometric titration of dilute ceric sulfate (0.1 to 2 mg.) and dilute potassium dichromate (17 to to 260 γ) solutions.

Quadrivalent uranium, prepared by passage of uranyl acetate (in $4M$ hydrochloric acid) through a silver reductor, was found by Belcher, Gibbons, and West (*10*) to be one of the most stable titrants for the direct titration of ferric iron.

IRON (II)–ETHYLENEDIAMINE TETRAACETATE AS A REDUCTANT. Belcher, Gibbons, and West (*11*) studied the effect of EDTA on the redox potentials of the iron(III/II) and copper(II/I) systems and found that the potential of each system was markedly reduced, being 0.12 and 0.13 volt *vs.* N.H.E., respectively. With the resulting enhancement of the reducing power of the lower valence state (resembles titanous), a variety of oxidants could be successfully titrated. Silver was determined reductometrically by Přibil, Doležal, and Simon (*118*) by titrating with iron(II)–ethylenediamine tetraacetate.

CYANIDE. The reaction between elemental sulfur and cyanide ion has been applied to the volumetric determination of sulfur in acetone extracts by Skoog and Bartlett (*145*).

CERIC TITRATIONS. Dilts and Furman (*39*) employed coulometric generation of ceric ion to determine ferrocyanide in the 1.7 to 62 μeq. range with an over-all accuracy within ±0.40%.

Titanium and mixtures of titanium and iron were determined coulometrically by Dilts and Furman (*40*) by initial reduction in a Jones reductor and subsequent oxidation with electrolytically generated ceric ion.

Small quantities of uranium(IV) in the presence of iron were determined by Hahn and Kelley (*57*) through oxidation with standard sulfatocerate using a platinum reference and gold indicator electrodes.

Ceric sulfate was found by Saeki and Nakano (*128*) to be an effective titrant for the direct titration of 2-methyl-1,4-naphthoquinone and hydroquinone. Sodium acetate causes low results.

The usefulness of the titration of antimony(III) with ceric ion in the presence of iodine monochloride catalyst has been confirmed by Takagi and Nakano (*152*). Platinum-rhodium or tantalum *vs.* silver gave well defined end points.

Laboratory preparation and standardization of cerium(IV) perchlorate for routine applications are described by Smith (*148*).

DICHROMATE. Mixtures of quinone and hydroquinone can be analyzed for both species by the method of Brauer and Staude (*15*). Quinone is selectively titrated by thiosulfate to a suitable potentiometric end point. After the reaction product is run through an anion exchange column to remove the quinone monosulfurate, the hydroquinone in the effluent is titrated with dichromate.

Organic substances whose carbon atoms are individually attached to oxygen atoms—e.g., carbohydrates—can be determined with dichromate, using the heat of dilution of concentrated sulfuric acid in place of external heating, according to Launer and Tomimatsu (*91*).

PERMANGANATE METHODS. Tellurium in dilute concentrations ($3 \times 10^{-5}M$) has been determined by Amin, Issa, and Issa (*5*) through potentiometric titration of tellurite with $10^{-4}M$ potassium permanganate. Issa and Awad (*66*) titrated tellurium(IV) with alkaline permanganate and obtained a potential break of about 180 mv. at the end point.

The estimation of bivalent lead ions based on their oxidation to the quadrivalent state by alkaline permanganate (forming lead tetroxide and manganese dioxide) has been proposed by Issa, Issa, and Abdul (*67*).

Singh and coworkers (*142*) found oxidation with alkaline permanganate useful for the analysis of iodide, iodate, cyanide, methanol, glycerol, phenol, and salicylic acid.

FERRICYANIDE METHODS. Diehl and Butler (*38*) replaced ammonia with ethylenediamine in the titration of cobalt(II) with standard potassium ferricyanide. A larger potential break at the end point results. Cobalt and manganese can be determined successively in the sample by this procedure.

A volumetric method for hydrogen peroxide, based on its oxidation to oxygen by potassium ferricyanide in strongly alkaline solution, has been investigated by Vulterin and Zyka (*168*). Hydrazine, hydroxylamine, and isonicotinoyl hydrazide were determined by Vulterin and Zyka (*169*), using direct titration with potassium ferricyanide in alkaline media.

Ferricyanide in excess iodide was titrated by Burriel, Conde, and Jimeno (*18*), using mercurous ion as an air-stable reductant.

BROMINATIONS. Aichenegg and Haynes (*4*) applied a direct bromination titration to determine *o*-cresol, 4-chloro-2-methylphenol, or 2-methylphenoxyacetic acid using a dead-stop end point.

A volumetric method for the analysis of synthetic acetic acid derivatives was developed by Capitani and Imperiale (*20*). Vinyl acetate was determined by bromination at 0° in acidic aqueous solution by the bromide-bromate method. Unsaturated aldehydes (*85*) can be determined even in the presence of saturated aldehydes by direct potentiometric titration with bromine in absolute methanol.

IODINE AND IODINE METHODS. Schreiber and Cooke (*131*) scaled down the ordinary coulometric titration techniques to microvolumes—e.g., 30 μl.—of solution. With this technique 0.1 to 7 γ of arsenic, for example, could be titrated with electrically generated iodine with an accuracy of 2 to 5%. A platinum wire indicator electrode *vs.* S.C.E. was used for detecting the end point.

Externally generated chlorine, bromine, and iodine were employed by Pitts, DeFord, Martin, and Schmall (*116*) for the coulometric titration of arsenite in 1-m.e.q. quantities. Excellent results were obtained using platinum-calomel electrodes.

Chloramine-B has been proposed by Singh and Singh (*143*) for the indirect determination of oxidants which liberate iodine from potassium iodide. The chloramine-B is used for titrating the liberated iodine.

Rowley and Swift (*127*) titrated oxidizing agents by the indirect method of liberating iodine from iodide with the oxidizing agent, adding a known quantity of thiosulfate to reduce the liberated iodine, and finally titrating the excess thiosulfate with coulometrically generated iodine.

Press and Murray (*117*) have titrated sulfide ion in solution by coulometric generation of iodine, employing an amperometric end point.

KARL FISCHER METHODS. A substantial gain in stability of Karl Fischer reagents was achieved by Peters and Jungnickel (*113*) by substituting methyl Cellosolve for methanol. The addition of ethylene glycol to the pyridine also improved the end-point response.

Sneed, Altman, and Mosteller (*149*) investigated the effect of tetraethyllead, aromatics, olefins, mercaptans, and oxidation inhibitors present in aviation gasoline on the determination of the water content. Only mercaptans in usually large concentrations seemed to interfere. Reed (*123*) has described a procedure for determining moisture in refrigerant-oil mixtures by the Karl Fischer method.

IODATE METHODS. Stone (*150*) found that iodide was quantitatively oxidized to iodine by iodate if chloroform was added to dissolve the iodine. The dead-stop end point was useful for practical titrations.

HYPOCHLORITE. Uranium was determined in the presence of titanium and beryllium by Sekerka and Vorlicek (*134*), who precipitated the uranium with ammonia in the presence of ethylenediaminetetraacetic acid, and titrated the ammonia in the precipitate with standard calcium hypochlorite.

CHLORATE. Erdey and Mazor (*45*) determined vanadium by reduction to vanadium(II) in a cadmium reductor and titration with standard potassium chlorate to vanadium(III). Iodine-iodide catalyst was added to eliminate the necessity for heating.

NITRITE. Sulfate has been determined by Keller and Munch (*77*) by a procedure whereby the sulfate is precipitated from an alcohol-water solvent as benzidine sulfate. The precipitate is then dissolved and titrated directly with standard sodium nitrite using a platinum-calomel or tungsten-calomel electrode system.

NONAQUEOUS SYSTEMS. Tomicek, Stodolova, and Herman (*159*) performed reductometric titrations in glacial acetic acid. The reducing power of the titrants, thiosulfate, vanadyl acetate, and arsenious trichloride, could be varied by addition of strong acid or base. Bromine, chromic oxide, permanganate, iodine monochloride, bromate, iodate, chloramine-T, and lead tetraacetate were determined.

ELECTROLYSIS. Nakano, Nonaka, Oba, and Takagi (*104*) determined cadmium ion by the unorthodox procedure of electrolyzing the cadmium into a mercury pool and measuring the resistance of the resulting cadmium amalgam.

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Acid-Base Titrations in Nonaqueous Solvents

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DURING the past 2 years the interest in acid-base titrations in nonaqueous solutions has continued to increase. Part of the Seventh Annual Summer Symposium on Recent Developments in Titrimetry, sponsored by the Division of Analytical Chemistry and ANALYTICAL CHEMISTRY, was devoted specifically to acid-base titrations in nonaqueous solvents. One session of the 1955 AAAS Gordon Research Conference on Analytical Chemistry discussed nonaqueous titrimetry.

The number of reviews in foreign journals has decidedly increased. Papers paralleling those published in this journal from 1951 to 1954 have been appearing in Japan. The application of acid-base nonaqueous and aqueous titrations to characterization problems has been noteworthy in the Scandinavian countries. Publications on the strengths of acids and bases and their correlation in nonaqueous and aqueous media have continued.

The scope of acid-base titrations has been broadened in three significant directions: the ability to titrate weaker bases by using acetic anhydride as a solvent, more practical methods for the titration of acids, and differentiating titrations of acids and bases whose strengths are close together by proper choice of solvents and titrants.

This review includes published information through September 1955 *Chemical Abstracts* and other readily available journals.

DIFFERENTIAL AND DIFFERENTIATING TITRATIONS

There is some confusion concerning the use of the term "differential" to describe a potentiometric titration with two or more inflection points. Cox (*30*) proposed the term "differential" in 1925 to describe a titration with two like electrodes in a concentration-type cell. This term was selected because the titration, in effect, gave the first differential, dE/dV (E = e.m.f.; V = volume). MacInnes and Jones (*108*) the following year described a differential titration apparatus. Moss, Elliott, and Hall (*115*) and Kirrmann and Daune-Dubois (*99*) give examples of differential acid-base titrations in nonaqueous solvents. The term differential is well established for describing this type of titration.

The term "differentiating" has been suggested to describe a titration in which two or more acids or bases are determined by one titration from two or more inflection points (breaks) in the potentiometric titration curve. A differential titration may also be a differentiating titration if two like electrodes are used in a concentration-type titration cell and two or more inflection points are obtained from acids or bases of different strengths.

THEORETICAL

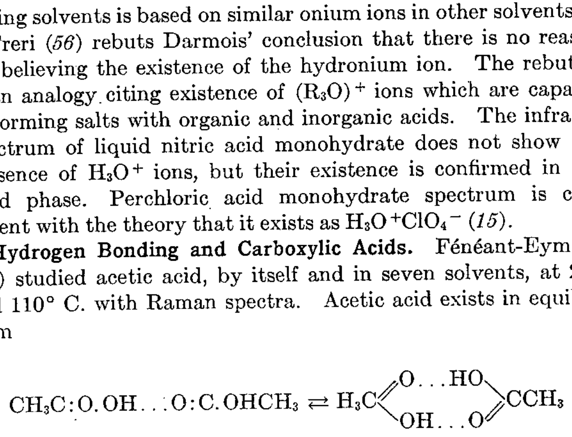
Concepts of Acids and Bases. Gutmann and Lindqvist (*74*) proposed a system of acid-base concept based solely on two solvent systems. These systems are: cationotropic solvosystems characterized by the ion migration taking place by means of cations and anionotropic solvosystems characterized by the ion migration taking place by means of anions. An acid, therefore, in a cationotropic solvosystem is a cation donor and in an anionotropic solvosystem is an anion acceptor. A base in a cationotropic solvosystem is an anion donor. Fundamentally Brönsted's prototropic solvosystem is a special case of the cationotropic system. The authors state that their concepts and definitions are not contradictory to the Lewis theory. It is pointed out that this theory has certain similarities to the theory of Usanovich but differs in that ionotropy excludes all complex ions as migrating units and also excludes redox reactions.

Gutmann and Lindqvist (*74*) remark that the weakness of the Lewis theory is the intended elimination of the solvent. They ignore the many reports of acid-base reactions in aprotic solvents in which it has not been demonstrated that the solvent takes any part in the reaction. They state that acids and bases are chemical concepts which have developed from observations in the field of chemistry and electrochemistry of aqueous solutions.

Onium Ion. Several articles have appeared confirming and denying the existence of the H_3O^+ ion. It is not that the H_3O^+ ion exists or does not exist that is of importance to quantitative acid-base considerations, but that our present concept of solvating solvents is based on similar onium ions in other solvents.

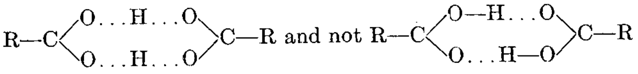
Freri (*56*) rebuts Darmois' conclusion that there is no reason for believing the existence of the hydronium ion. The rebuttal is an analogy citing existence of $(R_3O)^+$ ions which are capable of forming salts with organic and inorganic acids. The infrared spectrum of liquid nitric acid monohydrate does not show the presence of H_3O^+ ions, but their existence is confirmed in the solid phase. Perchloric acid monohydrate spectrum is consistent with the theory that it exists as $H_3O^+ClO_4^-$ (*15*).

Hydrogen Bonding and Carboxylic Acids. Fénéant-Eymard (*53*) studied acetic acid, by itself and in seven solvents, at 20° and 110° C. with Raman spectra. Acetic acid exists in equilibrium

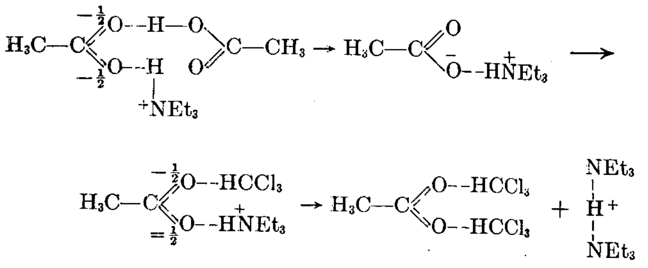


I II

with I predominating at 20° C.; I ⇌ II is endothermic. Harrand and Tuernal-Vatran (*75*) examined cinnamic and phenylpropiolic acids by infrared and Raman spectra and found complete absence of the C=O band. The dimer structure of carboxylic acid is given as



The reaction between acetic acid and trimethylamine in the nondissociating solvents carbon tetrachloride and chloroform were studied by infrared spectroscopy (*8*). It was found that the acetic acid dimer first hydrogen-bonded with one triethylamine molecule and then formed the salt as more amine was added. Carbon tetrachloride did not take any part in the reaction. Chloroform began to react when the stoichiometrical amounts of acetic acid and triethylamine were present to form the hydrogen bonded $(AcOH)_2.NEt_3$ and the reaction proceeded as



The equilibrium constant for the reaction AcOH + $NEt_3 \rightleftarrows$ $AcOH.NEt_3$ for 0.001*M* in carbon tetrachloride is 800 and in chloroform 8000.

Jander and Klaus (*82*) studied the role of water in the chemistry of acetic acid by potentiometric and conductometric titrations of perchloric acid with water. They concluded that water forms hydronium acetate $H_2O{:}H{:}OOCCH_3$.

Miscellaneous. Using thermodynamics of a dissociating solvent, Dunning and Dunning (*48*) conclude that if a certain species is present in a conventionally pure solvent as a result of dissociation, then no matter how complicated the equilibria may be that involves that species, $(\partial\mu_s/\partial m)_0$ of the solvent is zero. Addition of one of the dissociation products does not initially alter the chemical potential of the solvent. In higher concentrations the dissociation of the solvent is suppressed and the rate of change of chemical potential with concentration approaches normal behavior. Longster and Walker (*105*) studied the effect of permanent dipoles on the cohesive energy and solvent action of some highly polar substances. Schneider (*138*) presents the properties of the hydrogen bond in relation to the equivalent orbital representation of lone pair electrons.

The relationship between voltammetry and potentiometric and amperometric titrations is discussed by Kolthoff (*102*).

Grunwald (*73*) has considered the interpretation of data obtained by titrimetry in nonaqueous media. The activity coefficients of ionic species deviate greatly in media of intermediate or low dielectric constant. This deviation may cause inaccurate inflection points in potentiometric titrations or troublesome salt effects with color indicators. The deviations are best described by assuming the formation of ion pairs. The physical nature of the ion pairs, the magnitude of their dissociation constants, and the calculation of ion activity for partly associated electrolytes are discussed. Available generalizations are applied to one particular potentiometric titration, and methods are proposed for improving the accuracy of the inflection point. A quantitative treatment of media effects on color indicators for acid-base titrations is developed. For optimum results, the choice of an indicator is governed by the pH at the equivalence point and the solvent sensitivity indexes of the indicator and substrate.

STRENGTHS OF ACIDS AND BASES

Gold (*71*) formulates an acidity function, J_0, whose object is to provide a quantitative scale for the acidities of a medium toward secondary bases. The following definitions are given: $[R^+]/[ROH] = J_0K_{ROH}$; $J_0 = -\log j_0$, $J_0 = -pK_{ROH} + \log [ROH]/[R^+]$ where K_{ROH} is the constant expression of the strength of the base ROH. J_0 approaches Hammett's H_0 as H_0 approaches pH. Like H_0, J_0 is based on the experimental ionization rates of indicators.

Moiseev and Flid (*114*) propose an acidity function $y = \log C_{H^+} + pK_a + L\mu$ where $L\mu$ is a constant and μ is the ionic strength of the solution. *The function is related to* Hammett's H_0 by $H_0 = \log (C_{BH^+}/C_B) = pK_a - y$. This function is limited to H-acids or media in which H^+ is a dominant factor.

A "constant of acidity," corresponding to the dissociation constant, is proposed by Wolff (*167*). It is based on Brönsted's acidity theory and the existence of positive, negative, and neutral charged acids. The reaction of an acid on the base of another acid-base couple establishes a constant, *K*, of one couple to another, and by use of a reference couple a scale of acid strengths can be obtained. In dealing with dilute solutions, the concentration constant is used and in solvents of weak dielectric constant where ionization is very slight, a constant of definite acidity, as a function of total salt present, is used. The *K* of couples varies with the dielectric constant of the solvent. In each solvent there is a range of acidity between which all possible acidimetric reactions take place, this being determined by *K*.

An alternative mechanism of hyperconjugation based on the resonating semipolar single bond is proposed by Shamim-Ahmad and Wajid (*141*). This theory is used to explain qualitatively the shortening of bond lengths and the strengths of acids. Kabachnik (*87*) and Kabachnik and Ioffe (*88*) present the theory of tautomeric equilibrium and discuss the effect of the solvent on the acidity and the position of the keto-enol equilibrium. Values are given for the ionization constants for the keto and enol forms of some compounds.

Bates and Schwarzenbach (*10*) demonstrated that tedious and inaccurate extrapolation to zero ionic strength for the determination of true dissociation constants of organic acids can be avoided if spectral absorption data are combined with electromotive force measurements. Dissociation constants were obtained for three uncharged acids and three cationic acids.

Bates and Schwarzenbach (*11*) prepared three series of solutions: hydrochloric acid, an acetate buffer, and a triethanolamine buffer in which the water was successively replaced by ethanol. Hammett's acidity functions, H_0 and H_-, were determined with indicators, and the pH was measured in the conventional manner. The proton activity of the acetate buffer decreases with an increase of ethanol and increases in the triethanolamine series. In unbuffered hydrochloric acid, the proton activity decreases with removal of water and then increases, especially during the removal of the last traces of water. A much higher acidity is shown in ethanol than in water. The steric effects on the strength of acids and bases have been continued by Brown (*20, 21*).

The abnormally high ratio of the first and second ionization constants, characteristic of some dicarboxylic acids such as *cis*-caronic, maleic, diethylmalonic, and tetramethylsuccinic, can be explained by internal hydrogen bonding in the half-ionized form. McDaniel and Brown (*107*) state that evidence of such internal bonding can persist in aqueous solutions in competition with external hydrogen bonds to the solvent. The relative values of the equilibrium constants for very weak bases K_{BH^+} for the reaction $BH^+ \rightleftarrows B + H^+$ where B is the base, was found by Jaffé (*80*) to be $K_{BH^+} = K_{CH^+} ([B]/[C]) - [k/(k^0 - k)]$, where C is benzhydrol and K_{CH^+} is the acid dissociation constant of the conjugated acid C. The exceptionally high acidity of maleic and phthalic acids is attributed by Nash (*116*) to mutually antagonisic resonance and hydrogen bonding. Pauling (*122*) explains the magnitude of, and relation among, the dissociation constants of the various oxygen acids—e.g., boric, carbonic, hypochloric, chloric, and phosphoric—from the consideration of their structure.

Schaal (*134*) found, for indicators that ionize according to $AH \rightleftarrows A^- + H^+$, a function of the acidity, *H*, defined as $\log A_{-H^+} - \log \zeta_A = \log \zeta_{AH} = pK_a + \log (C_{A^-}/C_{AH})$. The ratio of C_{A^-}/C_{AH} was determined spectrophotometrically. A straight line relation exists between this function and the weight concentration of ethylenediamine and water. A 12% change in ethylenediamine corresponds to one unit of *H*. This function provides a means of measuring weak acids and was used for the determination of the strengths of several polynitrobenzenes (*135*).

Gero (*65*) points out that the following factors may be expected to have an important bearing on the base strength of polymethylenediamines. The inductive effect of the two nitrogen atoms on each other will mutually hinder their basic action. This effect increases as the number of carbon atoms separating them decreases. If one of the amine groups coordinates with a proton, its inductive effect on the other nonionic amino group will be greatly enhanced. A proton coordinating with one amine group in trimethylenediamine will hydrogen bond with the other amine group to form a stable six-membered ring. Ethylenediamine is the weakest base in this class. The base strength increases with increasing methylene groups separating the two amine groups, and at $CH_2 = 5$ the two amine groups are of not quite equal strength.

Vandenbelt, Henrich, and Vandenberg (*155*) determined pK_a values for substituted phenols, anilines, and benzoic acids by the absorption technique of Sager and coworkers and by the potentiometric method of Parke and Davis (*121*). Results from the two methods were in good agreement. The titration method is advantageous when there is more than one ionizable group in a molecule and when ionizations have minimum electron-displacing effect. The absorption method is preferable for relatively low pH values and for samples in which precipitation may occur at the neutralization point.

The rates of ionization of pseudo acids was studied by Pearson and Dillon (*123*). Plotting the logarithm of the ionization rate constant against the logarithm of the equilibrium constant divides the compounds into those with positive and negative deviations. As electron absorbers, $NO_2 > CO > CN \geq SO_2$. Transition from normal to pseudo acids is gradual.

The concentration of two weak monobasic acids, whose strengths are so close they give one break on the titration curve, can be determined by a method developed by Frisque and Meloche (*57*). The optimum pH at which the volume should be measured is defined as a function of the pK's of the two acids. This, together with the volume required for the total titration, allows the amount of each acid present to be calculated with minimum error.

Walter (*161*) found that the four-nitrogen porphyrin ring can simultaneously dissociate to give two protons. This porphyrin group participates in a series of acid-base equilibrium: $PH_4^{++} \rightleftarrows PH_3^+ + H^+$; $PH_3^+ \rightleftarrows PH_2 + H^+$. The intermediate, PH_3^+, is less stable than the PH_4^{++} or PH_2. Consequently the second proton is transferred more readily than the first. The ion, PH_4^{++}, is strong enough to be potentiometrically titrated in water.

The pK′ of substituted methylphosphonic acids was studied by Crofts and Kosolapoff (*33*). The pK′ for the second hydrogen is so close to that of the first that a differentiating titration cannot be made by chemical indicators. Halogen or hydroxyl group substituents on the methyl increase the strength of the first hydrogen. The pK'_{a1} and pK'_{a2} are given for a number of the substituted methylphosphonic acids. The effect of the sulfonic acid groups on aromatic systems was studied by potentiometric measurements and absorption in the 230- to 235-mμ range. Values of pK were tabulated for other than the SO_3H group. The applicability of Hammett's equation is discussed.

The acid dissociation constants of 16 ortho-substituted benzenephosphonic acids were determined by Jaffé, Freedman, and Doak (*81*). The pK values of 2,4- and 2,5-dinitrophenols are linear functions of mole fraction between 0 and 82% dioxane in water solution. Corey (*29*) found that *p*-nitrophenol is not appreciably ionized in pyridine but that 2,6-dinitro-3,4-xylenol and 2,5-dinitrophenol are partially ionized. Carboxylic acids are only slightly ionized, while 2,4,6-trinitrophenol is almost completely ionized.

Brockmann and Meyer (*18*) made a study of the potentiometric titration of the nitroaryl compounds, nitrobenzene, *m*-dinitrobenzene, picric acid, 2,4,6-trinitrophenetol, and 2,4,6-trinitro-*m*-cresol with sodium 2-aminoethoxide in ethylenediamine. Nitrobenzene does not titrate. The acidity increases with the number of nitro groups. The polynitro compounds are *L*-acids. The addition of one mole of base causes an electron shift of the nitro groups which reduces the electron density at the 1, 3, and 5 carbons. This makes it possible for a strong base to add to these positive centers. The acidity of nitroalkanes is due to H-acidity.

Sprengling and Lewis (*145*) report the dissociation constant, pK, for some phenols and methylolphenols determined by ultraviolet spectrometric methods. The 2,6-dimethylolphenols are hydrogen bonded within the molecule. The acid strengths of five phenols were determined in hydrazine (*160*).

Dulova and Vostrilova (*47*) determined the strengths of the following *H*- and *L*-acids in acetic anhydride: mono-, di-, and trichloroacetic, acetyl chloride and bromide, and arsenic(III) chloride, picric, and sulfosalicylic. These acids are weaker in acetic anhydride than in water but are slightly stronger than in acetone. The order of strengths differs from that found by Usanovich and Yatsimirskiĭ (*154*). The dissociation constants for several dibasic acids were determined in methanol (*50*); dithioöxamide is a monobasic acid, $K_a = 1.28 \times 10^{-11}$ (*170*).

The pK_a of acids and bases changes with a change of substituent groups in the molecule and with the solvents. Vandenbelt, Spurlock, and coworkers (*156*) studied the change of pK_a values for a number of compounds in aqueous solutions of methanol, ethanol, dimethylformamide, and dimethylacetamide. The concentration of water was varied from 20 to 100%. Kilpatrick and Fackenthal (*98*) determined the strength of phenylacetic acids in three solvents. The dissociation constants of cis and trans hydroxy-substituted cyclohexanecarboxylic acids in four solvents were measured by Kilpatrick and Morse (*96*). They also determined the dissociation constants of carbocyclic carboxylic acids (*97*). Additional information on the effect of formic acid on the strengths of acids has been reported by Shkodin and Izmaĭlov (*142*).

The Hammett acidity function, H_0, was determined at 20° C. for the system acetic acid–water (0 to 6%)–phosphoric acid (*101*). The apparent pK values at 25° C. were determined for 10 acids and bases in the solvent systems 2-methoxyethanol–water and dimethylformamide-water as a function of solvent composition (*143*). The van't Hoff factor, *i*, was determined for a series of homologous dicarboxylic acids in sulfuric acids and compared to the dissociation constants. Ludwig and Adams (*106*) determined the acidity function, H_0, for perchloric acid in the concentration range of 10^{-5} to $10^{-3}M$ in acetic acid containing 0 to 5% water.

DeVries and Gantz (*43*) made spectrometric studies of the dissociation constants of nitroguanidines, triazoles, and tetrazoles. The pK values were obtained graphically from curves plotted from absorption observations over the useful pH range. Dissociation constants too low (pK of 10 to 12) to be determined by potentiometric titration were easily measured. The values agree well with those predicted from electron-attracting tendencies of the substituents.

Rochlin, Murphy, and Helf (*132*) determined the pK values of a number of 1-substituted-5-aminotetrazoles in acetic acid and water. A number of other organic compounds not previously reported were included. The pK values of pteridine and 2- and 4-aminopteridine were determined spectrometrically (*42*).

The base strength of substituted pyrazoles varies with the nature and position of the substituent group. Veibel, Eggersen, and Linholt (*158*) studied these effects and determined the pK_b values in acetic acid. Kieffer (*95*) determined the pK_H of amines substituted by a phenyl group in the α or β position. Carotene was shown to be a proton acceptor (*163*), and its basicity was determined in relation to trichloroacetic and other acids.

The base strengths of four *cis-* and *trans-*1,2-aminoalcohols (*64*) and six nitrogenous bases in acetone and water (*152*) have been determined.

SOLVENTS

Fritz and Fulda (*59*) found that many weak bases that could not be titrated in acetic acid alone could be titrated in mixtures of acetic anhydride and acetic acid or acetic anhydride and nitromethane. Gremillion (*72*) independently found the same result. Fritz and Fulda limited their bases to those that do not react with acetic anhydride, while Gremillion found that by lowering the temperature to around 0° C. primary and secondary amines would not react sufficiently to cause an appreciable error in the determination.

Phenols are too weak as acids to titrate in water with potassium hydroxide or tetrabutylammonium hydroxide, but they can be titrated as weak acids in dimethylformamide or ethylenediamine. Carboxylic acids titrate as intermediate strong acids in water and dimethylformamide and as strong acids in ethylenediamine. Mineral acids are strong in the three solvents. Differentiating titrations are possible for mixtures of acid-type by selecting the proper solvents.

Chatten, Pernarowski, and Levi (*27*) investigated the reason why Fritz and Fulda (*59*) obtained erratic results for some heterocyclic nitrogen compounds when titrated in a mixture of acetic anhydride and nitromethane. It was found that bases whose pK_B in water was less than 11 reacted with the methylnitronic acid, and two breaks were obtained in the titration curve. (This is the expected reaction with primary or secondary nitroalkanes and any except the very weak bases.)

Tomíček and Vidner (*150*) found that acetic anhydride could be used successfully to remove water from formic acid. The e.m.f. change per tenfold change in concentration for 98% formic acid is 32 mv. For the anhydrous acid prepared by adding acetic anhydride the same value is 41 mv. A pH scale was established.

Using a dynamic vapor pressure method Swain and Brown (*147*) found that pyridine complexed with phenols and other acids. Pyridine and phenol forms 1 to 1 and 1 to 2 complexes. The equilibrium constant for the 1 to 1 complex was determined.

Dimethylformamide appears to be somewhat unstable in the presence of excess base (*39*). The catalytic effect of several substances on the hydrolysis of acetic anhydride has been studied (*5*). Levi, Chatten, and Pernarowski (*103*) found that Amerlite IRC-50 will remove the basic impurity for acetonitrile and that *p*-dioxane treated with the ion exchange resin would not discolor for at least 4 weeks when used to prepare a perchloric acid titrant. Anhydrous ethylenediamine may be prepared by removing the water as the benzene azeotrope (*31*). A method is given for the purification of formamide (*38*).

TITRANTS

The number of papers in this field is limited. Davis and Hetzer (*37*) found that diphenyl phosphate is a strong acid in aqueous and nonaqueous solvents. It can be used where perchloric acid or sulfuric acid is not applicable—i.e., in solutions of aprotic solvents. It is stable in solution and is sufficiently soluble in a large number of organic liquids for use as a titrant.

Organic Indicators. Davis and Hetzer (*37*) tested 17 indicator dyes for use with diphenyl phosphate and have arranged them on a potentiometric titration curve of the acid with sodium methylate as a titrant. Fritz and Fulda (*59*) prepared a chart of five indicators, methyl violet, α-naphthylbenzein, triphenylcarbinal, neutral red, and dibenzolacetone, that can be used in the solvent system nitromethane–acetic anhydride (4 to 1). The transition range of the indicators are blocked over the electromotive force of the glass-calomel electrode system. After a potentiometric titration of a substance has been made in the solvent with perchloric acid and the e.m.f. determined for the equivalent point, the proper indicator may be selected from the chart.

Gautier and Pellerin (*62*) found that tropeoline OO could be used interchangeably with methyl violet for titrating heterocyclic alkaloids. For a differentiating titration of the two basic nitrogens of the alkaloid phytostigmine, tropeoline OO changes color when $E = M$ and crystal violet when $E = M/2$.

Michaelis and Mizutani (*113*) studied the effect of alcohols upon the pH at which indicators change color. The pK values of several indicators in the nitrophenol series were determined. The pK values increased as the amount of alcohol in the solvent increased. *p*-Nitro-*p*-aminoazobenzene was found to be a good indicator for the titration of phenols in ethylenediamine (*149*). Tomíček and Vidner (*150*) found that malachite green, gentian violet, neutral violet, safranin, and orange I were indicators suitable for use in formic acid.

Potentiometric. A discussion of the fundamental concepts of electrode potentials is given by De Bethune (*41*). The standard potential of the silver–silver chloride electrode was determined by Bates and Bower (*9*) from 0° to 95°. Kirrmann and Daune-Dubois (*99*) use the platinum-platinum differential electrode system of MacInnes for differentiating titrations of acids of different strengths in formamide with aqueous 0.5*N* potassium hydroxide. Mantzell (*109*) presents a series of tests and arguments to prove that electromotive force measurements made with the antimony electrode are not doubtful as indicated by Hauke.

Brockmann and Meyer (*17*) found that platinum electrodes rather than antimony could be used as differential electrodes for differentiating titrations of acidic groups of polyacids. Glenn (*67*) modified the silver–silver chloride reference electrode to eliminate its instability in the vicinity of the end point when used in conjunction with the glass electrode. Dubois, Maroni, and Walisch (*45*) discuss the experimental methods and applications of the dead-stop end point method while Dubois and Walisch (*46*) extend its scope to the titration of acids and bases.

Mercury-glass electrodes can be used for titrations in acetic acids but they are slower in reaching equilibrium than the calomel-glass electrodes (*118*). Ohlweiler and Meditsch (*117*) found that the smooth platinum-glass electrodes could be used, but they are not so satisfactory as the calomel-glass system.

High Frequency. An electromagnetic method for determining the end point is described by Jensen (*84*). High frequency titrations can be used for the titration of acids in dimethylformamide with sodium methylate (*40*). Sharp titration curves were obtained for the ammonium ion and for strong acids over a range which included concentrations as small as 0.0001*M*.

Photometric and Others. Photometric detection of the end point promises to be an important aid to the analytical chemist in the future. Most of the work reported has been in aqueous solution using modified standard photometric equipment. The technique is easily usable in nonaqueous systems. The Beckman DU spectrophotometer can be readily adapted to photometric titrations (*100*). An automatic coulometric titration apparatus with photometric detection of equivalence point is described by Wise, Gilles and Reynolds (*166*).

The principles of photometric titration, its advantages and disadvantages, factors affecting its accuracy, and apparatus and technique are discussed by Goddu and Hume (*70*). Polarization titrations, principles, execution, and applications are described by Franck (*54*). Low frequency alternating current is used, and the equivalence point is recognized by a change in polarization resistance of a suitable indicator electrode It is applicable to most ordinary titrations.

Reilley and Schweizer (*131*) report a photometric method of titrating in the ultraviolet region of the spectra where the molar absorptivities of many substances have their maximum value. The method requires only small volumes of solution. Titration of several bases were made in acetic acid with perchloric acid.

By the addition of an absorbing species of weaker basicity it is possible to titrate a substance whose acid or base form absorbs in the ultraviolet region.

METHODS

Fritz and Fulda (*59*) studied the addition of acetic anhydride to acetic acid for potentiometrically titrating bases with perchloric acid. For most tertiary amines and some alkali metal salts the addition of acetic anhydride increased the sharpness of the break and the rise in potential at the end point. The use of nitromethane–acetic anhydride often gave sharper end points than the acetic acid–acetic anhydride mixture. The increased $\Delta E/\Delta V$ was ascribed to the complete removal of water. Glass and calomel electrodes were used for the potentiometric titration, but organic indicators may also be used.

Almost simultaneously Gremillion (*72*) found that bases too weak to titrate in acetic acid could be titrated in a mixture of acetic acid and acetic anhydride. Tertiary amines and amides, such as urea and allylthiourea, gave adequate potentiometric breaks for analytical purposes. The reaction between acetic anhydride and primary and secondary amines is retarded at 0° C., so that very weak amines of these classes may be titrated.

Schiff bases can be titrated in acetic acid with perchloric acid (*55*). A larger break is obtained in acetonitrile using perchloric acid in *p*-dioxane as the titrant. Mixtures of the parent aliphatic amine and Schiff bases can be titrated with separate breaks in chloroform using the same titrant. Aromatic amines and their Schiff bases can be titrated in acetonitrile.

Pifer, Wollish, and Schmall (*128*) found that differentiating titrations could be made of a number of strong bases (in acetic acid) in a solvent of 1 part of acetic acid and 10 parts of chloroform. Perchloric acid in *p*-dioxane was used as the titrant. Good inflections were obtained for the acetates of sodium and potassium, lithium and potassium, sodium and ammonium, and lithium and ammonium. Alkali metal salts can also be differentially titrated in the presence of organic bases. Potassium and ammonium acetates show nearly the same basic strength in the acetic acid–chloroform solvent so could not be differentiated.

Deal and Wyld (*39*) found that potassium hydroxide and 2-propanol could be used to titrate weak acids in dimethylformamide or ethylenediamine without taking the rigorous precautions required by the Moss, Elliott, and Hall method (*115*). Caldin and Long (*23*, *24*) measured the equilibrium constant for the system $OH^- + EtOH \rightleftarrows OEt^- + H_2O$. In a solution made by dissolving sodium or sodium hydroxide in ethanol containing x weight % water, the values of x and the percentages of base present as OEt^- are, respectively: 0.2, 99; 0.5, 98; 1.0, 96; and 2.0, 94. This explains why the addition of 1% water has little effect on the rate of reaction between OEt^- and trinitrotoluene or similar substances in ethanol solutions.

Sensi and Gallo (*140*) made an extensive study of the titration of 65 basic nitrogen compounds of various nitrogen configuration in acetic acid with and without the addition of acetic anhydride. Weak bases, such as nicotinic acid, gave a weak inflection point in acetic acid but a sharp inflection point when 10% acetic anhydride was added. Sodium *p*-aminosalicylate titrated with one very weak inflection point and one fair inflection point in acetic acid but titrated with a single strong inflection point when acetic anhydride was added. It is noteworthy that Gremillion (*72*), Fritz and Fulda (*59*), and Sensi and Gallo (*140*) independently and at about the same time discovered that when acetic anhydride was added to acetic acid the inflection point was materially inhanced.

A procedure employing anionic surface-active agents for determining quaternary compounds was developed by Carkhuff and Boyd (*25*). Sodium lauryl sulfate is used as the titrant. A large number of pharmaceutical preparations may be analyzed by this procedure. Das and Palit (*36*) report further applications of G-H solvent (propylene glycol–chloroform, 1 to 1) in the titration of organic bases.

Gautier and Pellerin (*63*) studied the alkalimetry of organic base sulfates in nonaqueous solvents. Acetates of calcium, barium, zinc, manganese, lead, and mercury were titrated by Das (*35*) in G-H solvent both potentiometrically and with an organic indicator.

A unique method for the estimation of ethylenes has been reported (*34*). The ethylenic compound is mixed with a known mercury(II) acetate in methanol. The mercury(II) acetate reacts with the ethylenic compound and the excess is titrated in G-H (propylene glycol–chloroform, 1 to 1) solvent with hydrochloric acid in the same solvent to the thymol blue end point. One mole of the acetate is equivalent to 2 moles of hydrochloric acid. The addition product is equivalent to 1 mole of acid. The difference between blank titrations of the mercury(II) acetate and the sample is a measure of the ethylenic compound.

Papers on titrations in liquid ammonia have appeared (*164*, *165*).

A micromethod for differentiating titrations of polybasic acids in ethylenediamine has been devised by Brockmann and Meyer (*17*). The method is used to characterize the number and type of acidic groups in antibiotic-type fermentation products. From the number and position of the inflection points, together with other information, the molecular weight of the compounds is established.

The phenolic acidity of phenolic esters was titrated potentiometrically or to an indicator end point by Glenn and Peake (*68*) in ethylenediamine with potassium methoxide. Phenolic esters of carboxylic acids undergo aminolysis in excess ethylenediamine at ambient temperatures producing phenols and amides of ethylenediamine. Lactones of phenols also undergo aminolysis. The carboxylic acid may be either aliphatic or aromatic and the phenol either benzenoid or naphthenic. The titration determines only the phenolic groups originally present or those in the aminolysis product.

A direct method for the determination of carboxylic acid anhydrides in carboxylic acids has been developed by Johnson and Funk (*85*). Morpholine reacts with the anhydride to produce equimolar quantities of an acid and an amide. The reduction in the amount of morpholine in the sample compared to the amount in the blank, when titrated with hydrochloric acid in methanol, is a direct measure of the anhydride. The method is equally adaptable to the determination of amounts of anhydride from about 0.01 to 100% with good accuracy and precision. The titration may be made potentiometrically or to the methyl yellow end point. *N*-Carboxy-α-amino acid anhydrides react in the same manner as carboxylic acid anhydrides.

Berger, Sela, and Katchalski (*12*) titrated *N*-carboxy-α-amino acid anhydride with sodium methylate in the presence of methanol to the thymol blue end point. The method can be used to follow the polymerization of α-amino acid anhydrides.

A method developed for the analysis of the insecticide allethrin (*79*) is applicable to the determination of esters of β-keto alcohols and for the determination of acid anhydrides in the presence of the parent acid.

High molecular weight ketones in the presence of free fatty acids can not be determined by the standard hydroxylammonium chloride method. Metcalfe and Schmitz (*111*) modified the method as follows: Hydroxylammonium chloride is dissolved in a mixture of 2-propanol and methanol (65 to 35). A measured excess of octadecenylamine in 2-propanol is added to facilitate complete reaction between the ketone and the hydroxylammonium chloride. The excess amine is titrated with hydrochloric acid in 2-propanol to the bromophenol blue end point. Under these conditions free fatty acids do not effect the titration.

Sulfuric and hydrochloric acids and their mixtures were differentially titrated conductometrically in acetic acid with several alkali acetates by Higuchi and Rehm (*77*). Marcus and Winkler

(*110*) titrated conductometrically nitric acid in a mixture acetic acid and acetic anhydride with potassium acetate.

LEWIS ACIDS

Silicon tetrachloride is unusual in the paucity of addition compounds it forms. It acts as an acid in benzene, acetone, or ether and will react with amides (*66*). Two covalent compounds have been isolated with the formulas: $SiCl_4.10HCONH_2$ and $SiCl_4.5HCON(CH_3)_2$.

Hirshon, Gardner, and Fraenkel (*78*) present evidence of the existence of radicals in the presence of Lewis acids by studying the paramagnetic resonance absorption of bianthrone, fluorenone, and similar compounds in sulfuric acid. The same compounds, when heated with anhydrous aluminum chloride and cooled, showed no paramagnetism. Cryoscopy has been applied to volumetric analysis by Usanovich, Sumarokova, and Nevskaya (*153*). Cryoscopic titrations were made of pyridine with tin(IV) chloride in dichlorobenzene and of a mixture of pyridine and quinone with tin(IV) chloride. The results appear to contradict the principles of cryoscopy based on the homogeneity of the solution and the precipitation of pure solvent.

The qualitative order of acidity of 19 acids, 3 *H*-acids, and 16 *L*-acids was determined by Hawke and Steigman (*76*) in benzene, chlorobenzene, and chloroform. Pease and Luder (*124*) report a continuation of this study of the nature of *L*-acids. They present conductance data for the systems composed of the acids aluminum bromide and tin(IV) chloride and the bases pyridine and quinoline in thionyl chloride.

Somayajulu and Palit (*144*) studied the *L*-acid character of aryl and alkyl iodo compounds in enhancing the solubility of naphthalene in hydrocarbon solvents. Dupuy and Nortz (*49*) reversed the usual procedures and titrate the "titrant," a standard tellurium tetrabromide solution, with the organic base to be analyzed. A few drops of concentrated solution of copper(II) chloride or cobalt(II) bromide in ethanol act as the indicator. Tellurium tetrabromide forms colorless complexes with an organic base. The method is also applicable in the presence of aliphatic acids, alcohols, etc.

SPECIFIC APPLICATIONS

Owens and Maute (*119*) adapted micro and semimicro nonaqueous techniques to the determination of trace amounts of weak and strong organic bases and organic acids in acrylonitrile. Both potentiometric and indicator methods were used. Choline hydroxide can be determined in concentration of 0.002 weight % with a variation generally less than 0.001 weight %. Acetic and acrylic acids can be determined in concentrations of 0.002 weight % with a variation generally less than 0.001 weight %. Acetic and acrylic acids can be determined with a precision of 0.001 weight % and weak bases with a precision of 0.0015 weight %.

A method for the determination of the amounts of sulfuric acid mixed with either hydrochloric or nitric acids using only acid-base titrimetric procedures was developed by Critchfield and Johnson (*32*). A potentiometric or visual indicator titration of the mixed acids is made in acetonitrile with morpholine in acetonitrile as the solvent. The hydrochloric or nitric acid and one half of the sulfuric acid titrate at the first potentiometric inflection point. Methylene blue or methyl yellow indicators can also be used for hydrochloric acid–sulfuric acid mixtures. For nitric acid–sulfuric acid mixture a potentiometric method must be used. Variations of these methods are given. The effects of concentration, water, and other acids are discussed.

A rapid determination of carboxyl end groups in the polyester, polyethylene terephthalate, has been devised by Pohl (*129*). The procedure involves dissolving the polymer in benzyl alcohol at a high temperature, quickly mixing the solution with chloroform, and then titrating with sodium hydroxide to the methyl red end point.

Kaye (*94*) applied the method of Fritz and Lisicki to the analysis of hexahydro-1,3,5-trinitro-*S*-triazine (RDX) and its content in explosive compositions.

Proguanide [*N*-(*p*-chlorophenyl)-H^5- isopropylbiguanidine] can be potentiometrically titrated in acetic acid with perchloric acid in the presence of mercury(II) acetate. Acetic anhydride enhances the inflection point (*1*).

Semimicrodeterminations of benzoyl groups have been made by saponifying with either 9*N* sulfuric acid or 5*N* sodium hydroxide, extracting the benzoic acid with chloroform, and titrating with sodium methylate to the thymol blue end point (*136*). Warner and Haskell (*162*) determined the quantities of acids and bases in tobacco smoke. The acids were potentiometrically titrated in acetic acid or acetic acid and cyclohexane. The construction of an antimony electrode is described.

Gremillion (*72*) determined the sulfuric acid catalyst concentration in continuous industrial ethanol esterification mixtures composed of varying amounts of ethanol, acetic acid, ethyl acetate, and water. An equal volume of acetic anhydride is added to the sample, and the sulfuric acid is titrated with sodium acetate in acetic acid. Sulfuric acid titrates as a monobasic acid.

The method of Fritz and Lisicke, with slight modifications, has been extended to the analysis of wool, wax, and fatty acids (*130*). Meyer (*112*) studied some characteristics of fillers and pigment by titration, in conjunction with the iodine number. Nitric acid in a mixture of acetic acid and acetic anhydride was determined by conductometric titration with potassium acetate (*110*). Antimony microelectrodes were used (*89*) to follow the titration of naturally occurring fatty acids in alcoholic solvents with potassium hydroxide. The method was applied to potentiometric chromatographic determinations of steric and oleic acids. Ganguly, Mukherjee, and Ghosh (*60*) found that clays and certain resins could be characterized in nonaqueous solvents.

Pharmaceutical Applications. Doernberg, Hubacher, and Lysyj (*44*) determined phenolphthalein in chocolate preparations by a modified Moss, Elliott, and Hall procedure. A simple enclosed titration system is described using antimony-glass electrodes. The phenolphthalein is extracted from the chocolate preparation with carbon tetrachloride, the extract is dried, and the residue is transferred to a titration flask. Pyridine-ethylenediamine (5 to 1) is added, and the solution is titrated with sodium 2-aminoethoxide in pyridine.

Ryan, Yanowski, and Pifer (*133*) titrated barbiturate salts in dimethylformamide with sodium methoxide to the thymol blue end point to determine the free acidity. Barbiturate salts, in either bulk or pharmaceutical preparations, were extracted with an appropriate solvent from a sulfuric acid–acidified solution, the solvent was evaporated, and the free barbituric acid was dissolved in dimethylformamide and titrated.

Streptomycin, dihydrostreptomycin, and framycetin and their salts, including the sulfate, have been titrated in 1,2-ethanediol by adding an excess of perchloric acid and back titrating with potassium acid phthalate. Sulfate is precipitated with 95% equivalent of benzidine before the titration (*125*).

Aspirin and acetophenetidine (phenacetin) were determined in the presence of caffeine in A.P.C. tablets (*168*). The aspirin was extracted from the acidic medium with a chloroform-ether (1 to 3) mixture. The solvent was evaporated, dissolved in dimethylformamide, and titrated with lithium methoxide in benzene-methanol solution. Phenacetin is hydrolyzed to phenetidine hydrochloride and extracted from an alkaline solution with chloroform and titrated with perchloric acid.

Ephedrine can be determined in oily and aqueous nasal sprays by dissolving in 50 ml. of chloroform and titrating with perchloric acid (*26*). Caffeine and caffeine citrate in pharmaceutical preparations were titrated directly by Pernarowski (*126*) in acetic acid with perchloric acid to the α-naphtholbenzein end point. Kashima (*90–93*) studied the analysis of drugs in acetic acid [see also (*3*)]. Codeine phosphate in the presence of acetylsalicyclic acid, phenacetin, and caffeine can be titrated with perchloric acid (*127*). Diphenan (4-benzylphenyl carbamate) and urethane can be determined by heating with 0.1*N* perchloric acid for 0.5 and 1 hour, respectively, and back-titrating with triethylamine. Barbiturates in pharmaceutical preparations dissolved in chloroform and poly(ethylene glycol) 400 can be rapidly and accurately titrated with sodium methoxide (*148*).

Stockton and Zuckerman (*146*) found that not only could sodium *p*-aminosalicylate be analyzed in G-H solvent, but that the extent of decomposition to *m*-aminophenol could be determined. The method is applicable for the assay in pharmaceutical preparations as well as to the hydrated or anhydrous salts.

EQUIVALENT WEIGHT AND IDENTIFICATION OF ORGANIC COMPOUNDS

The development of simple techniques for isolating a compound by its functional group and determining the equivalent weight is one of the major advances in qualitative organic analysis. Berger (*13*) has shown that the potassium alkyl xanthogenates can be titrated in acetic acid to an indicator or potentiometric end point, even though they decompose; the AcO^- neutralizes a free H^+, thus keeping the reaction stoichiometric. Alcohols are converted to the potassium xanthogenates and the equivalent weight is determined. He further reports that the equivalent weight of an alcohol may be determined by preparing the xanthogenate and titrating a portion of the recrystallized, dried, and weighed material in acetic acid with perchloric acid. Crystal violet indicator or potentiometric titration may be used. The average accuracy for the 12 alcohols reported was within 0.4%.

Berger (*14*) also found that the equivalent weight of a carboxylic acid may be determined quickly by converting the acid to the *S*-benzylthiuronium derivative, recrystallizing, drying, weighing, and titrating in acetic acid with perchloric acid. The titration may be made using crystal violet indicator or potentiometrically. The method is applicable to impure acids, and to esters, salts, amides, and nitriles after the acid has been obtained by the appropriate method. The average deviation of the equivalent weight found for the 16 acids reported was 0.4%.

Schotte and Veibel (*139*) found that tertiary alcohols exhibited anomalous behavior when treated with thiourea in alcoholic solution. The tertiary alcohol is shaken with concentrated hydrochloric acid and converted to the chloride, which is then converted to the *S*-benzylthiuronium chloride. The *S*-benzylthiuronium chloride is converted to the picrate for characterization.

Jurecek and Vecera (*86*) developed a method for identifying alkyl and haloalkyl groups attached to oxygen or nitrogen. The compound is heated with hydroiodic acid and the alkyl iodide formed is converted to thiuronium iodide and then to the picrate. The picrate is titrated in acetic acid.

Clark and Wang (*28*) isolated the amines as the picrates in the usual manner, recrystallized, dried, weighed, and titrated with perchloric acid in acetic acid to the methyl violet end point. Primary, secondary, and tertiary aliphatic and aromatic amines may be determined. The method is accurate [see also (*2*)].

Lewenz and Serijan (*104*) condensed aromatic hydrocarbons of the series, benzene, mono-, di-, and trialkylbenzenes with phthalic anhydride and determined the melting point and neutral equivalent of the resulting *o*-aroylbenzoic acids.

Veibel, Kjaer, and Plejl (*159*) studied the potentiometric titration of some 1-aryl-5- or 3-pyrazolones with sodium hydroxide in ethanolic solution. Isomeric 3- and 5-pyrazolones were prepared and titrated. The titrations showed that the tendency to enolization was less for the 3-pyrazolones than for the 5-pyrazolones. However, the titrations curves were of two distinct types and were useful for determining if an unknown pyrazolone belonged to the 3- or the 5-series.

MISCELLANEOUS

Blaedel and Knight (*16*) give a method of purification and properties of the disodium salt of ethylenediaminetetraacetic acid for use as a primary standard. The chemistry of organic perchlorates is discussed by Burton and Praill (*22*). The hazards of these substances are of interest to analytical chemists because of the conditions of formation.

Tubbs (*151*) devised a method for the determination of the potentiometric titration inflection point by use of concentric arcs. A series of concentric arcs is inscribed on a clear plastic sheet, which is superimposed upon the curved portion of a titration curve to find the arc which most closely approximates that of the titration plot. A dot is made at the template center through a hole in the plastic. A straight line is drawn through the two dots resulting from the operation which intersects the steep portion of the curve at the inflection point.

A modification of the Schmall extractor for extracting salts of organic acids and bases has been made (*137*).

REVIEWS AND BOOKS

"Non-Aqueous Titration" by Palit, Das, and Somayajulu (*120*) devotes 22 of 122 pages to theoretical discussion. The remainder of the book is concerned chiefly with titration in glycolic (G-H type) solvents. A brief discussion of titrations in other solvents is given. Gautier (*61*) discusses the nature of acids and bases, effect of solvent on strengths, and practical applications. The possibilities of photometrically following the course of the titration of a weak acid, or base, with a strong base, or acid, are reviewed by Goddu and Hume (*69*) and compared to potentiometric methods.

Many reviews of specific and general interest have been published: correlation of methods of identification of organic compounds by titration of their derivatives (*157*); current status of analysis by titration (*52*); pharmaceutical applications with emphasis on barbituric acids (*51*); indicators and tables of indicators to use with specific compounds (*7*); theoretical aspects (*6*); selection of the medium for a particular titration (*58*); applicability of titration in nonaqueous media to pharmaceutical products (*169*); a review of the preparation, stability, reproducibility, and standard potentials of the silver–silver halide electrodes in aqueous and nonaqueous media (*83*); the meaning and significance of steric effects (*19*); and the applications of acetic-perchloric acids to titrations of basic compounds (*4*).

ACKNOWLEDGMENT

The reviewer wishes to express his gratitude to Emory E. Toops, Jr., for assistance in preparing this review.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Chromatography and Electrochromatography

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IN THE 2 years following the last review in this series, there have been extensive advances in the instrumentation and application of chromatographic methods of analysis, and there have been corresponding refinements, modifications, and applications of the analogous electrochromatographic methods. There have also been significant increases in our understanding of the principles underlying both of these analytical procedures (*31, 35, 89, 96*).

Much of this current progress is readily available in articles in the chemical, physical, medical, and biological journals. Some 1000 applications are tabulated each year in *Chemical Abstracts*. A large number of articles of biological and biochemical significance are summarized in *Excerpta Medica*. Many additional applications, that are reported in various specialized fields, remain untabulated. A large number of specialized reviews and several bibliographies citing hundreds of applications have also been prepared. As a typical example, a single review describing the applications of the specialized partition chromatography in the highly restricted field of steroids includes some 436 citations (*39*). The second edition of Henley and Schuettler's bibliography of electrophoresis and electrochromatography, completed March 1, 1955, contains 3193 entries (*40*) (see sections about books on chromatography and electrochromatography).

In addition to the reviews and bibliographies, a number of books cover various aspects of chromatography and of electrochromatography. Some of these books have treated the historical aspects of the subjects (*41, 57, 104*), many have included basic operational procedures, but most have been devoted to the uses of chromatographic and electrochromatographic procedures for the examination of mixtures (*9, 35, 64, 66, 68, 96, 107*). In virtually all the original applications of the chromatographic procedures, the primary interest was centered in the substances being examined rather than in the basic principles of the methods that were employed.

As a consequence of this practical point of view, there are still many misconceptions concerning the basic requirements for chromatography and for electrochromatography. There is by no means universal agreement regarding the basic arrangements, conditions, and operations required for chromatographic and electrochromatographic separations. Few books and reviews attempt a classification of the various modifications of the several basic techniques. Indeed, in some treatments, a single property such as the nature of the sorptive reaction—adsorption, for example—provides an arbitrary basis for restriction of the subject matter. In other treatments, another unrelated property such as the nature of the migration medium—paper, for example—has served for restriction and orientation of the field.

As a result of these arbitrary restrictions, there have been few extensive comparisons and evaluations of techniques. Moreover, most of the practical applications have been based upon empirical advances that have been perpetuated and extended by analogy.

OBJECTIVES OF THIS REVIEW

Because of the few attempts at systematic classification of chromatographic and electrochromatographic methods, and because of the availability of practical applications in summary articles and specialized books, this report elaborates many of the variable aspects of chromatographic and electrochromatographic methods. It illustrates the interrelationships among these variable conditions, and it treats many of the conditions that are important in theoretical as well as in practical considerations of the technique. This presentation incorporates the principles of differential migration, driving forces, and resistive forces that were elaborated in this series of reviews. To facilitate readability and to conserve space, it restricts current citations to books and reviews wherever possible.

The preservation of perspective has necessitated reference to many facts established before the period covered by this review. Most of these facts are so well known or can be so readily found in the current books that specific citations are not required.

DIFFERENTIAL MIGRATION METHODS

All the analytical methods considered in this review fall in the category of differential migration analysis. These methods may be classified with respect to the relative size of the initial zone of the mixture. As described before for chromatography, and as indicated by Figures 1 to 3 for electrical migration, the degree of resolution of a mixture depends largely upon the size and arrangement of this initial zone. The most effective differential migration techniques for the resolution of complex mixtures depend upon the use of initial zones that are small or narrow relative to the length of the migration system, as indicated by Figures 1 to 7.

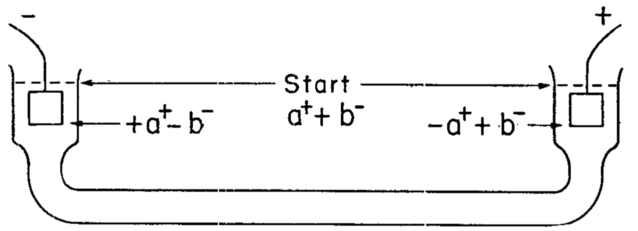

Figure 1. Schematic arrangement for examination of mixtures by differential electrical migration in an unstabilized or stabilized solution

Dependent upon their properties and upon the composition of the electrodes, ions may be estimated in the electrode compartments or as deposits on the electrodes. The arrangement is analogous to the chromatographic frontal analysis with the collection of a large sample. It permits detection of a change in the composition of the mixture, but it rarely leads to the isolation of a single component from multicomponent mixtures.

Chromatography is the most widely applicable, differential migration method of analysis. The differential migration method next in importance from the standpoint of applicability is electrochromatography, followed by continuous electrochromatography.

These three differential migration methods have many features in common. All depend upon the selective action of driving and resistive forces, which are summarized in Tables I and II. All depend upon the differential migration of the components of mixtures from a narrow zone in stabilized media. All provide the components of the mixture as discrete zones in the migration media. These methods usually provide the components of the mixture in their original condition without chemical change or alteration. In all these methods, the separated substances must be detected and estimated by appropriate tests which may be applied to the migration medium, to extracts of the medium, or to the effluent from the systems (see section on detection of separated substances).

DEFINITIONS

The definition of chromatography or chromatographic analysis proposed by Williams and Weil and quoted in recent books (*9, 104*) is ambiguous, because it applies to other separatory methods such as fractional crystallization, solvent extraction, and distillation as well as to chromatography. In this laboratory, the following definitions have been employed for chromatographic analysis.

Chromatographic analysis is a widely applicable, differential migration technique in which flow of solvent or gas causes the components of mixtures to migrate differentially from a narrow initial zone in a porous, sorptive medium.

Electrochromatographic analysis is a differential migration technique in which direct current electrical potential causes the components of mixtures to migrate differentially from a narrow initial zone in a stabilized, background electrolytic solution.

At first sight, differential electrical migration from a wide zone in a pH gradient appears to be an exception to this definition (Figure 4). But consideration of the distribution of the separated substances in the migration medium shows that, even with this method, migration from a narrow zone may be more effective than migration from a wide zone (see section on electrochromatography).

A number of descriptive names continue to be used synonymously with electrochromatography and its modifications. The most common are: zone electrophoresis, paper electrophoresis, and ionography (*64, 94*).

Continuous electrochromatographic analysis is a differential migration technique in which flow of background electrolytic solution through a wide porous medium and transverse, direct current electrical potential cause the components of mixtures to migrate differentially from a narrow continuous stream of the solution flowing contiguously with the background electrolytic solution.

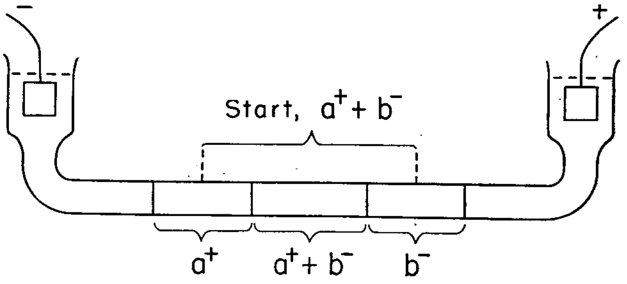

Figure 2. Schematic arrangement for examination of a mixture of cations and anions by differential electrical migration from wide zone of mixture in an unstabilized or stabilized background electrolytic solution (not shown)

This arrangement with stabilization media is analogous to the chromatographic frontal and trailing analysis with a wide initial zone of the mixture. It permits separation of but a small portion of the fastest migrating cation and anion. This arrangement and several of its modifications, all without stabilization media, form the well known moving boundary method and the differential electrophoresis technique (*59, 64*).

These definitions are in agreement with the concepts, procedure, and nomenclature proposed by Tswett in 1906. They indicate that formation of a chromatogram by migration from a narrow zone of the mixture is a critical step in the analytical procedures. They eliminate all the sorption and partition techniques that depend upon migration from a wide zone of the mixture (*93*).

DISCOVERY OF CHROMATOGRAPHY

There is by no means universal agreement regarding the origin and evolution of chromatography. On the one hand, some contend that migration of a wide zone (as in Figure 2) constitutes the chromatographic procedure. Accordingly, the early uses of columns with flow of solution by D. T. Day represent the discovery of columnar chromatography, and the utilization of paper strips and sheets with flow of solution by F. F. Runge represents the discovery of paper chromatography (*3, 104*). On the other hand, there are some who hold that formation of a chromatogram from a narrow initial zone (as in Figure 3) represents the basic chromatographic procedure as described by Tswett and as indicated by the definitions given above. Consequently, the early uses of columns and of paper sheets and strips with flow of solution but without formation of the chromatogram with fresh solvent do not represent the discovery of chromatography (*93*). Until these divergent views are reconciled, there cannot be agreement regarding the discovery of chromatography.

CLASSIFICATION OF CHROMATOGRAPHIC METHODS WITH RESPECT TO VARIABLE CONDITIONS

Chromatographic methods are commonly classified with respect to a variety of variable conditions that are known to affect the separations. But the very large number of these conditions and their multiple effects have precluded a simple systematic classification.

From nearly every standpoint, the mixture to be examined is the most important variable in chromatographic analysis. It may be classified with respect to its physical conditions: gas, liquid, solute, and solid. Gases and vapors may be examined chromatographically using another gas to form the chromatogram. This procedure is the basis of the recently developed, rapid, effective, gas chromatography (*23, 38, 48, 62*). Solutes are usually examined by conventional chromatography with flow of wash liquid to form the chromatrogram. Liquids and solids must be vaporized or dissolved before they can be examined.

Mixtures may also be classified with respect to the polarity and reactivity of the components. The components may range from nonpolar or weakly polar hydrocarbons and symmetrically halogenated hydrocarbons to strongly polar organic acids and bases and various ionized inorganic substances. Even the nonionized weakly polar substances differ in their reactivity at interfaces. This variation of the sorbability of virtually all substances is attributed to variations in polarity that are related to the atomic structure (*21, 56, 95*) or to the molecular structure (*9, 27, 96*). Many of the nonionized substances may be converted into derivatives and ionized complexes of various kinds (*34*). Ionized substances may be converted into ionized complexes with the same sign of the electrical charge; they may be converted into un-ionized complexes; or they may be converted into complexes of opposite charge (*6–8, 21, 56, 88, 89, 91, 92, 95*). These changes alter the polarity and the sorbability of the components of the mixture, and they affect the separability.

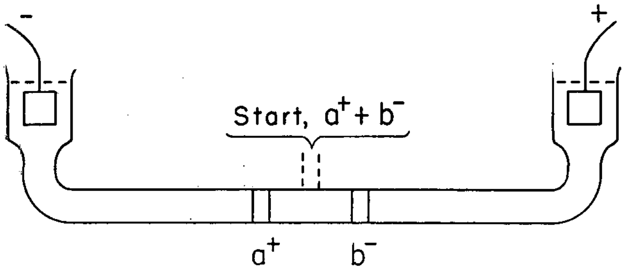

Figure 3. Schematic arrangement for examination of a mixture of cations and anions by differential electrical migration from a narrow initial zone of mixture in an unstabilized or stabilized background electrolytic solution (not shown)

This arrangement with stabilization media is analogous to chromatographic analysis with a narrow initial zone of the mixture. It permits the separation of a multicomponent mixture into all its components (*59, 64, 93*).

The solvent employed for the dissolution of the mixture is another critical variable condition in chromatography. The solvent must dissolve the mixture, and it must permit the components to be held in the sorptive system (*16*). For empirical investigations, preliminary tests of solubility are usually made with weakly polar solvents. If the mixture does not dissolve, solvents of greater polarity are employed until the mixture dissolves. But if partition chromatography with reversed or inversed phases is to be employed (*71*), it is often desirable to employ a very polar solvent for dissolution of the mixture.

The wash liquid for formation of the chromatogram may be the same as the solvent for the mixture, or it may be another liquid of slightly greater polarity (*69*). A gradual increase in polarity may be obtained by the selection of a series of increasingly polar liquids, or by the addition of increasing quantities of a polar solvent or acidic or basic solutions to the wash liquid (*74*). Several mechanical devices have been designed to facilitate the gradual addition of the more polar liquids (*2, 10*). Various reagents and complex-forming substances are occasionally added to the wash liquid to increase its eluting power.

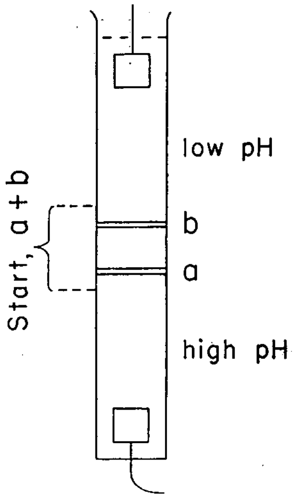

Figure 4. Schematic arrangement, with wide initial zone, for examination of a mixture of ampholytic substances with isoelectric points corresponding to particular regions in pH gradient lying between sections with high and low pH

Solutes contained in the initially wide zone migrate from both directions toward the region of their isoelectric point and concentrate in narrow zones (*53–55*). For the degree of separation see Figure 6.

Both the solvent and the wash liquid must be selected with respect to the properties of the sorptive phase as well as the properties of the mixture. Both the solvent and the wash liquid must permit the dynamic reversible distribution of the dissolved mixture between the solvent and the sorptive phase. Neither the solvent nor the wash liquid should dissolve or alter the porous sorptive medium.

Even though solvent, wash liquid, and sorbent are selected so that the components of the mixture are sorbed reversibly, the separability of a particular mixture often varies a great deal from one sorbent and solvent combination to another. This variation of separability of selectivity is a function of solvent and sorbent. Many combinations of solvent and sorbent may serve for the resolution of a particular mixture (*8, 21, 32, 36, 37, 95*). Many other combinations may not be effective, even though all the other requirements are fulfilled. This variation is so great that there are few simple rules for the prediction of the selectivity of a particular system.

Elutriants for the recovery of separated substances are frequently the wash liquids themselves. If the sorptive system with each separated substance can be separated mechanically, the sorbed substances may often be eluted rapidly with very polar solvents or with acidic or basic solutions.

The sorptive medium is variable in many ways. With respect to physical form, the sorbent may be a fine powder (usually employed in columns), a granular mass (also employed in columns), a porous solid, or fibrous sheets, strips, and pads (as paper and chamois). The sorbent and its sorptive properties vary with its chemical composition, its method of preparation, and its method of treatment or activation (*72*). The sorptive process may vary with the physical and chemical properties of the sorbent, as may be seen from Table I.

Table I. Driving and Resistive Forces Employed in Chromatographic Systems

Driving forces	
Flow of gases	Nonselective
Flow of liquid (solvent and wash liquid)	Nonselective
Resistive forces	
Sorption at interface, between	
Gas-solid	Selective
Gas-liquid	Selective
Liquid-solid	Selective
Liquid-liquid	Selective
Sorption by fixed liquid, between	
Gas–fixed liquid	Selective
Liquid–immiscible fixed liquid	Selective
Sorption by ion exchange substance, between	
Gas–ion exchange resin	Selective
Gas–ion exchange zeolite	Selective
Liquid–ion exchange resin	Selective
Liquid–ion exchange zeolite	Selective
Sorption by reversible chemical reactions, between	
Liquid–chelating agent, etc.	Selective

The principal classification of sorbents as surface-active adsorbents (Table II), as ion exchange substances (*43*), and as fixed liquid or partition sorbents is usually an oversimplification. With a single sorptive medium, such as silicic acid, the sorptive process may differ a great deal, depending upon the solutes and the solvent (*50*). For example, hydrated silicic acid and moist Celite may be employed in gas adsorption chromatography and in gas partition chromatography (*28, 38, 48, 62*); and they may also serve in conventional adsorption chromatography, partition chromatography, and ion exchange chromatography.

One of the major classifications of chromatography is based upon the form of the migration medium—namely, columns and paper which provide columnar chromatography and paper chromatography (*94*). As intimated already and as indicated by many books (see section on books about chromatography), this classification serves for the practical restriction of many aspects of the subject. It is, however, highly artificial. As an example, the paper itself may be rolled and utilized in the form of a column (*20*), the cellulose fibers may be separated and packed into a column (*86*), or cellulose fibers may be twisted into a sorptive thread. In all four arrangements—paper, rolled paper column, packed cellulose column, and sorptive thread—the sorptive properties are all the same.

Chromatographic methods may be classified with respect to the arrangement for flow of the wash liquid. Common arrangements for flow are: upward, downward, linear or one-way, radial, spherical or segmental, and transverse in succession or two-way. In paper especially, there may be irregularities in the flow and in the distribution of the solvent (*1, 105*) and these may vary with the solvent itself (*1*).

The flow rate of the wash liquid is another important variable. With sorbents of large permeable particles, such as the ion exchange resins, the flow rate must be slow enough so that the solutes have time to penetrate the particles and to diffuse out again as fresh solvent flows by. With very small particles, the flow rate may be very much faster than with large particles (*92*). With sorbents of impermeable particles, as with powdered sugar, the solutes do not penetrate the particles; hence, even with large particles, the flow of solution may be very rapid. As an example, the chlorophyll and carotenoid pigments extracted from green leaves may be separated in a few minutes with petroleum ether as the solvent. With an impermeable surface-active sorbent such as powdered sugar, the separation of a mixture by flow of solution is as rapid as the separations effected by gas chromatography (*23, 38, 48, 62*).

Numerous attempts have been made to devise chromatographic methods that operate continuously. But most of these are arranged so that the sorbent moves countercurrent to the flow of mixture, providing separation of only the most sorbed or least sorbed or so that the sorbent is regenerated in successive batches (*18, 43, 45, 70*).

In view of all these variables, the classification of chromatographic methods has become extremely involved. This complexity has led Giddings and Eyring to conclude in their theoretical considerations (*31*), that "The general problem of chromatography is, in a practical sense, not solvable."

DETECTION OF SEPARATED SUBSTANCES

Much attention has been devoted to the detection and estimation of substances separated by chromatography. In principle, at least, very minute quantities may be separated as discrete zones by chromatographic methods. So far as has been tested, the minimum quantities that form discrete zones lie at or below the limits of their detectability (*95*). Separated substances may be detected in the chromatographic system in treated or clarified systems (*5*), in extracts of portions or sections of the chromatographic system, or in successive portions of the effluent from the chromatographic system. One of the advantages of gas chromatography is the convenient detectability of the separated substances in the effluent wash gas (*23, 38, 48, 62*).

Table II. Driving and Resistive Forces Employed in Electrochromatography

Driving force	
Direct current electrical potential	Selective or nonselective
Resistive forces	
Properties of background electrolytic solution	
Hydration or solvation	Selective or nonselective
Viscosity	Selective or nonselective
Complex formation	Selective
Chelate formation	Selective
Properties of stabilization medium	
Sorption at interface, between	
Liquid-solid	Selective
Sorption by fixed liquid, between	
Liquid-liquid	Selective
Sorption by ion exchange substance, between	
Liquid–ion exchange resin	Selective
Liquid–ion exchange zeolite	Selective

Detection methods may be placed in four major categories, dependent upon the nature of the detection process: (1) nuclear reactions as with radioactive tracers and neutron activation analysis (*21, 74, 95*); (2) physical properties, as fluorescence (*57*), phosphorescence, spectral absorption (*58*), emission spectra, isotope additions or dilutions, migration rate relative to solvent (R value), migration sequences, etc.; (3) chemical properties, usually reactions that convert the separated substances into colored or fluorescent products; and (4) biological methods, as growth stimulation, growth inhibition, and various stimulation effects. Most of these detection methods have been reported in the articles dealing with particular applications of the chromatographic methods. Many of them are summarized in the books that are cited in this review.

APPLICATIONS IN VARIOUS FIELDS

Owing to its adaptability, the chromatographic technique continues to find wide application in many fields. In the biochemical and biological fields of carotenoids, chlorophylls, steroids (*39*), nucleic acids, amino acids, and antibiotics and in many physiological phenomena such as respiration and photosynthesis, there is no substitute for chromatography as an exploratory analytical tool. Summaries of many of these investigations are available in handbooks, such as "Methods of Biochemical Analysis" and "Methods in Enzymology." A series of review articles dealing with principles, gas-liquid chromatography, practical aspects of paper chromatography, quantitative determination of amino acids, inorganic substances, organic acids, peptides, carbohydrates, porphyrins, nucleotides, thyroid hormones, antibiotics and vitamins, steroids and sterols, proteins, amino acid metabolism, and separation of amino acids with ion exchange resins has appeared in the *British Medical Bulletin* (*13*).

Ion exchange columnar chromatography is being widely utilized for the separation of organic and inorganic substances, both cations and anions (*43*). It is playing an indispensable role in the separation and isolation of the thiourea complexes (*7*); the rare earths (*21, 88, 89, 91, 92*), and the transuranium elements (*21, 95*). Surprisingly, the anion exchange resins serve for the sorption and separation of many cations in acid solutions (*56, 95*). Anion exchange columns have been utilized for the separation of phosphoric acids (*44*), organic acids from alcohol (*16*), serum proteins (*11*), and amino acids (*46*).

The isotopes of nitrogen contained in ammonium ions have been separated by sorption on a cation exchanger (*90*). There is indication that the isotopes of carbon (C^{14}), contained in glycine or in alanine, may also be separated (*74*).

Columnar ion exchange chromatography has been employed for the desalting of sea water (*70*) and for many other purposes that are reviewed by Hiester (*43*). It has been utilized with solvent pH gradients (*74*).

Columnar chromatography has been utilized with various sorbents such as boric oxide plus silicic acid (*15*), methyl Cellosolve plus silicic acid (*109*), silicic acid plus Celite, (*50*), and rolled paper (*20*), and with various solvents (*69*).

Devices for automatically stopping the flow of solvent (*42*), for preparing solvent mixtures (*2, 10*), and for collecting fractions of effluent under vacuum (*52*) have been described.

Paper chromatography has been modified in many ways principally by variation of the shape of the paper (*17, 28, 29, 67, 76, 83*), by operation on a small scale (*97*), and by fixation of various polar liquids (*14, 71*). The flow of solvents into paper has been studied (*1, 105*), and a compact method for two-way development (*108*) has been described. The effect of solvents on separations (*32, 36, 37*) and ion exchange in the paper itself (*82*) have been examined. New fluorometric (*57*) and direct spectrophotometric methods (*58*) have been reported, and a concentration procedure with the "ring oven" (*101*) shows promise of wide application. Special applications include rare earths (*60*), various inorganic ions (*37*), alkali and alkaline earth ions (*32*), chelates of various ions (*6, 8*), amino acids (*36, 63*), blood (*79*), fatty acids (*71*), plant growth substances (*51*), and forensic science (*61*).

Of particular significance is the separation of D,L-histidine into two distinct zones. The D form migrates more slowly (*100*).

BOOKS ON CHROMATOGRAPHY

Following the comprehensive book on chromatography by E. and M. Lederer and the specialized book on inorganic paper chromatography by Pollard and McOmie, both of which were cited in the last review in this series, there have now appeared three monographs concerning various aspects of paper chromatography. Hais and Macek (*35*) have prepared a comprehensive volume on the uses of paper chromatography. This publication, prepared with the assistance of 21 collaborators, contains 3795 literature references. Turba (*96*) has published an exhaustive treatise on the chromatography, countercurrent extraction, and paper electrophoresis of proteins. The introductory portions of this book include an extensive treatment of the various modifications of paper chromatography. Block and Zweig have prepared a new edition of their manual on paper chromatography, and Durrum has added a chapter on paper electrophoresis (*9*).

Williams (*104*) has revised his attractive little monograph on the elements of chromatography. This revised publication presents a concise elementary introduction to the subject. As indicated already, the viewpoint regarding the nature of chromatography, the definition of chromatography, and the discovery of chromatography are not in accord with those of Tswett, that are elaborated in this review (*93, 94*).

Hesse and Weil (*41*) have translated Tswett's first paper on chromatography from the original Russian, and they have added notes on this publication and on Tswett's life as well. In this paper in 1903 Tswett had recognized the importance of the formation of a narrow initial zone of the sorbed mixture before washing with fresh solvent. Three years later in his article in the *Berichte der deutschen botanischen Gesellschaft*, Tswett called this washing of the narrow zone of the sorbed mixture with fresh solvent "the formation of the chromatogram" (*93*).

The applications of paper chromatography in botany have also been summarized (*85*).

VARIABLE CONDITIONS OF ELECTROCHROMATOGRAPHY

Electrochromatographic methods, with few exceptions (*75*), are modifications of the arrangement shown in Figure 3. They are usually less variable than the analogous chromatographic methods. Nevertheless, the number of variable conditions is still large, and some of these conditions have several effects upon the migrations.

Mixtures to be examined must be in solution. For most separations, the components of the mixture should be charged or ionized, or they should be convertible into ionized products or complexes before or after addition to the background electrolytic solution.

Solvents for dissolution of the mixture may be the background electrolytic solution itself, various electrolytic solvents, or even nonelectrolytic organic solvents. As a rule, the ionic concentration of these solutions should not exceed that of the background electrolytic solution. The solvents should also be selected so that a precipitate is not formed when the zone of the mixture is added to the background electrolytic solution.

The background electrolytic solution must be selected so that it conducts a suitable amount of electrical current without overheating. The conductivity of the background electrolytic solution may be adjusted by variation of the electrolyte and its concentration. This background electrolytic solution must also be selected so that the components of the mixture remain in solution and migrate selectively. It is usually an aqueous solution of acids, bases, or salts. At potentials of about 10 volts per centimeter, the concentration of this solution may vary from about 0.05*M* or less (*81*) for strongly dissociated acids, bases, and salts, to 1*M* or more for a weakly dissociated base such as ammonia (*24, 25, 59, 64*). Complex-forming reagents may be added to the solution to facilitate the selective migrations (*34, 78*).

Stabilizing media may be viscous solutions such as dilute agar and methyl Cellosolve (*77*), agar gels (*33*), powders such as starch (*26*), powdered glass (*12*), cellulose (*86*), and ion exchange resins (employed in columns or in troughs), and fibrous materials such as string (*4*) and paper (employed as pads, strips, or sheets) for one-way (*19, 22, 24, 25, 30, 34, 49, 65, 78, 80, 81, 84, 98, 102, 103, 106*) or two-way migration (*22*). Usually the stabilizing medium is selected so that the components of the mixture are not sorbed or are but weakly sorbed. Sorption of the mixture may affect the selectivity of the migration system, and it may produce certain undesirable effects such as trailing of the migrating zones (*78*).

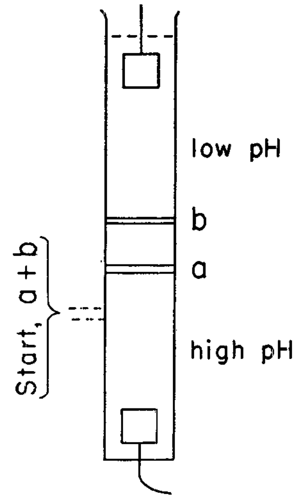

Figure 5. Schematic arrangement, with narrow initial zone, for examination of a mixture of ampholytic substances with isoelectric points corresponding to particular regions in the pH gradient lying between sections with high and low pH

Solutes contained in the narrow zone migrate unidirectionally until they reach the region of their isoelectric point. With this arrangement, the narrow initial zone may be placed at any point in the gradient (*47, 65*). For the degree of separation see Figure 7.

As indicated by the selected references, the most widely employed stabilizing medium is paper. But the paper of different grades varies greatly in thickness and in density (*1, 25, 65, 106*). Moreover, different lots of the same grade of paper have been found to differ with respect to the isoelectric point and the electro-osmotic flow (*24, 25*). The migration system may vary in length from a few centimeters to about 2 meters (*65, 78*).

Many devices for the support of the moist paper have been described (*19, 24, 25, 30, 65, 78, 80, 84, 106*). With some devices, the paper is stretched horizontally in an inert moist atmosphere (*64*). With others, it is draped over a glass rod or suspended between two glass rods (*30*). It may also be suspended in a bath of nonconducting, water-immiscible organic solvent (*80*), or placed between glass or plastic plates (*24*). It may even be wrapped in polyethylene sheets and placed on a water-cooled Thermopane window (*78*).

Electrodes, usually of graphite, platinum (*106*), or even copper (*98*), may be placed in contact with the moist paper, or they may be placed in the electrolytic solution in separate vessels (*24, 25, 59, 64, 68*). With the former arrangement, the electro-osmotic flow is small; with the latter arrangement, the flow is larger, varying with the pH of the electrolytic solution and the isoelectric point of the paper (*25, 106*). The hydrostatic pressure equivalent to the electro-osmotic pressure is extremely small (*25*). Zones of easily detectable substances such as hydrogen peroxide have been utilized to determine both the electro-osmotic flow and the hydrostatic flow of the background electrolytic solution (*24, 25, 106*).

The electrical potential may vary from a few volts to 100 volts per cm. (*80, 81, 102, 103*). For most investigations, a potential of 5 to 10 volts per cm. is employed, and the paper is cooled. But with very dilute background electrolytic solutions, the conductivity is so low that potentials of as much as 100 volts per cm. may be applied without cooling the moist paper (*81*).

With potentials of about 10 volts per cm. and with migration periods of a few hours, migration distances are usually only a few centimeters. With migration periods of a day or more, the migration distances may be a meter or two.

ELECTROCHROMATOGRAPHIC APPARATUS

Most research workers have constructed their own electrochromatographic apparatus. Consequently, there have been many designs and modifications to suit particular aims and objectives.

In the past 2 years, the electrochromatographic method has proved extremely useful as a routine analytical, clinical, and chemical tool. This has prompted attempts to standardize the apparatus and procedure, and it has stimulated the apparatus manufacturers to produce various models. These products are now readily available in England, France, Germany, Japan, Sweden, and the United States.

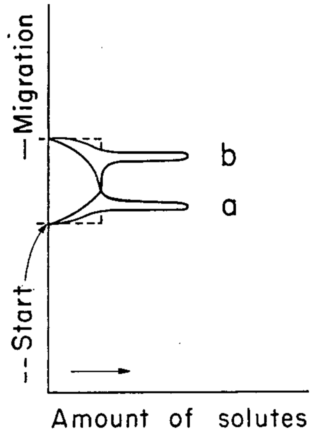

Figure 6. Schematic distribution, from wide zone in pH gradient, of two substances, *a* and *b*, with isoelectric points corresponding to particular regions in gradient

This distribution of the solutes corresponds with the separations illustrated by Figure 4. The width of the concentrated zones and of the diffuse portions will vary with the width of the initial zone, mobilities, pH gradient, and many other variable factors.

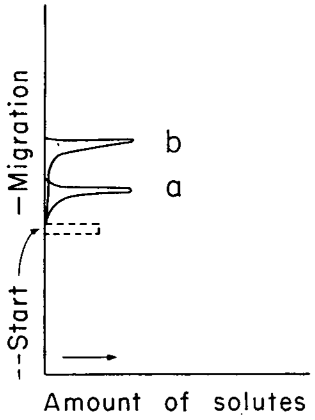

Figure 7. Schematic distribution, from a narrow zone in a pH gradient, of two substances, *a* and *b*, with isoelectric points corresponding to particular regions in gradient

This distribution of the solutes corresponds with the separations illustrated by Figure 5. The width of the concentrated zones and of the diffuse portions will vary with the width of the initial zone, the mobilities, the pH gradient, and many other variable factors (*47, 65*).

DIFFERENTIAL ELECTRICAL MIGRATION IN A pH GRADIENT

Kolin (*53–55*) has shown that a pH gradient stabilized by a density gradient is an effective medium for the separation of ampholytic substances with isoelectric points lying within the range of the gradient. The arrangement is indicated schematically in Figure 4, and the differential electrical segregation of the solutes in the initial wide zone is shown in greater detail in Figure 6. With this arrangement, the several zones are not separated very far from one another; hence a special pipet has been designed to remove the zones separately (*54*). The effect of a density gradient has also been examined (*87*).

This use of a pH gradient with ampholytic substances has been simplified through use of a narrow initial zone of the mixture and through use of paper as a stabilizing medium (*47, 65*). The basic arrangement is shown in Figure 5, and the distribution of the solutes after migration is indicated in Figure 7.

DETECTION OF SEPARATED SUBSTANCES

Most of the methods employed for the detection of substances separated by electrochromatography are identical with those employed in chromatography, particularly in paper chromatography (*99*). The principal categories of these detection methods with illustrative examples have been presented above.

As in chromatography, the rates of migration in electrochromatography may be made the basis for the description and the identification of the components of mixtures. These rates are variable, however, with many different conditions such as the background electrolytic solution, the paper, and the wetness of the paper. They must also be corrected for the electro-osmotic displacement (*24*). As in conventional chromatography, the electrochromatographic sequences may be employed as the basis for description and identification (*78*).

Because of the utilization of chromatographic and electrochromatographic methods with various substances at very low concentration, there is increasing need for a highly sensitive, widely applicable detection method. But it is unlikely that a universal, sensitive detection method will be found.

APPLICATIONS OF ELECTROCHROMATOGRAPHY

Electrochromatography, particularly the one-way and the two-way modifications, have found extensive application in biochemical investigations. These methods have proved particularly effective with proteins, when the migration conditions are selected so that there are minimum sorption and denaturation. These methods have also proved effective with biological products such as peptides, amino acids, carbohydrates, flavins, enzymes, and growth hormones (*9, 40, 49, 59, 64, 68, 73, 86, 96*).

Electrochromatographic methods are finding use with inorganic substances such as those found in living organisms as well as those encountered in the inorganic world (*59, 64, 68*). Thus far, however, a systematic scheme of electrochromatographic analysis for the elements has not been worked out. They may prove to be useful with isotopes, as indicated by countercurrent electromigration (*75*).

Most of these applications of electrochromatography have been described in connection with the preparation and purification of various substances. Many uses have been tabulated in recent bibliographies and books (see discussion of books on electrochromatography).

CONTINUOUS ELECTROCHROMATOGRAPHY

The continuous procedure for the resolution of mixtures by combination of electrical migration with transverse flow of solution has been applied to the purification of various proteins and radioactive tracers (*9, 12, 59, 64, 68, 96*). Thus far, this technique has been employed primarily as an analytical tool. Preliminary considerations of solution requirements, of dilution factors, and of maintenance costs indicate that operation of this method on a large scale would be costly unless solution recovery could be effected economically.

Apparatus for continuous electrochromatography has now been assembled by several manufacturers in England, Sweden, and the United States. As more workers become familiar with its requirements and possibilities, this separatory technique is certain to be adopted for many routine preparations.

BIBLIOGRAPHIES AND BOOKS ON ELECTROCHROMATOGRAPHY

Most of the studies and applications of electrochromatography have been tabulated in the second edition of Henley and Schuettler's "Electrophoresis Bibliography" (*40*). In a short review, Parker (*73*) has cited some 240 articles. Additional reviews are included in the books on paper chromatography by Turba (*96*) and by Block, Durrum, and Zweig (*9*).

McDonald and his collaborators (*64*) have prepared an extensive book on differential electrical migration in stabilized media, with emphasis upon the physical and chemical aspects of ionography and upon applications in various fields. This monograph also contains an excellent summary of the evolution of the electrochromatographic methods.

Lederer (*59*) has written an introduction to electrophoresis and related methods. This treatment is from the practical point of view. It includes numerous selected examples of separations in various organic and inorganic fields, and contains a carefully selected bibliography.

Mori and Kobayashi (*68*) have prepared an extensive, well-illustrated monograph in Japanese on practical paper electrophoresis.

Wunderly (*107*) and Macheboeuf, Rebeyrotte, Dubert, and Brunerie (*66*) have written monographs concerned with the clinical aspects of paper electrophoresis.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Inorganic Gravimetric and Volumetric Analysis

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IN CONTRAST to previous practice the present review of inorganic gravimetric analysis makes no claim to complete coverage of relevant publications. Regrettably, a rather large number of significant contributions have been omitted, but it is the authors' hope that they have succeeded in covering researches of relatively wide interest and, in general, of examining these more critically.

Undoubtedly, the reports of more general interest deal with homogeneous precipitations and with attempts to relate structure and formation of analytical reagents. However, in spite of the interesting and promising work which has been done in the latter field, the chemist who seeks precipitation and color reagents finds as yet no acceptable rule which leads him to reagents not already familiar. There is also a growing interest in the field of gravimetry. It is almost inevitable that some of the precipitants, rejected too hastily as analytically unacceptable, should be restored to the list of useful reagents by minor adjustment of conditions.

The review of volumetric analysis covers publications recorded in *Chemical Abstracts* since January 1952. Although reference is made to approximately half of the papers published during this period, the authors recognized a great deal of good research in articles not included in this review. It is hoped that those chosen are of general interest and indicate the direction of progress in inorganic volumetric analysis.

GRAVIMETRIC ANALYSIS

METHODS OF SELECTIVE SEPARATIONS AND GENERAL GRAVIMETRIC REAGENTS

Taimni and Agarwal (*93–95*) have introduced a rather fertile approach to separations in studying the precipitation of the sulfide group. They used an acid decomposition of thio salts prepared with sodium or ammonium sulfide. Data were given for the gravimetric determination of arsenic, selenium, tellurium, molybdenum, and tin. In another paper (*96*) procedures were recorded for the determination of bismuth, cadmium, lead, and indium. In the determination of bismuth, the solution was treated with ammonium hydroxide to a turbidity, and then ammonium sulfide was added. The precipitate was dried at 105° to 115° C. In the case of cadmium the precipitating reagent was sodium sulfide added in small quantities. The excellence of the results obtained with all four metals encourages the hope that the authors will investigate the applicability of their methods to other metals where sulfide precipitation is particularly useful.

The growing applications of trithiocarbonic acid as a precipitation reagent were extended by Pilz (*75*) to the separation of iron sulfide from aluminum and titanium in a tartrate medium; 8-quinolinol can be used to separate aluminum and titanium in the filtrate. The general precipitant dithiocarbamate, "sodium parcarbate," in the presence of alkali cyanide quantitatively precipitates only lead and thallium. Premanis, Schaible, and Bergner (*79*) recorded a procedure for isolating lead in this manner in the presence of eight associated cations. The white precipitate was dried at 110° to 130° C. Cadmium and mercury interfered.

Following their work on tetraphenylarsonium chloride as an analytical reagent, Willard and Perkins (*102*) examined the analytical applications of the tetraphenyl derivatives of phosphorus and antimony. Because of the insolubility of tetraphenylstibonium chloride, its value was restricted principally to the precipitation of permanganate ions. A rather serious difficulty was the lengthy drying period required for constant weight. In general the ions precipitated by these two reagents are much the same as those precipitated with the arsonium derivatives. Interest in these reagents will be enhanced by the fact that the precipitates are soluble in chloroform. Platunov (*76*) has continued his investigations of the applications of naphthoquinoline chloride. Both alpha and beta derivatives may be used for the gravimetric determination of tungsten and, in the presence of excess thiocyanate ion, for the determination of cadmium and zinc.

Various derivatives of benzoic acid have been used successfully as gravimetric reagents. Crepaz and Marchesini (*27*) used *m*-nitrobenzoic acid for the quantitative separation of trivalent iron from various bivalent cations. Under the same conditions precipitates were formed with chromium, thorium, copper, lead, mercury, aluminum, titanium, tin, barium, uranium, zirconium, and a few rare earth oxides. Majiimdar and De (*65*) used 5,6-benzoquinaldinic acid for the determination of zinc at pH 2.85, cobalt at pH 3.24, nickel at pH 3.00, and manganese at pH 2.90. The salts could be dried at 110° to 115° C., and weighed as the hydrated salts. Gastinger (*41*) stated that adsorbed cupferron dissolved with greater difficulty than the precipitate itself; hence, the metal cupferronates must be ignited. Optimum conditions for quantitative precipitations were described. The 2-fluorenyl analog of cupferron described by Oesper and Fulmer (*71*) is more sensitive than cupferron for the precipitation of iron. Copper interfered if present in amounts greater than the iron. Bhatki and Kabadi (*17*) stated that 2-naphthylthiourea under various conditions yielded precipitates with the following cations: silver, lead, mercury(II), bismuth, copper, cadmium, cobalt, and nickel. Most of the heavy metals could also be precipitated by the sodium salt of 3,5-dibromoanthranilic acid.

2-Naphtholaldoxime (*69*) and 2-hydroxy-1-naphthaldoxime (*36*) have been recommended for the precipitation of copper, nickel, and cobalt. Berges (*16*) used 2-methyl-8-quinolinol to separate copper and magnesium. Copper was precipitated below pH 4, and magnesium above pH 10. An interesting communication was made by Hollingshead (*54*) on the selectivity of certain derivatives of 8-quinolinol with respect to chelation of some Group IIIB elements. 2-Methyl-8-quinolinol chelated with gallium, indium, thallium, and yttrium, with yttrium precipitating partially at pH 5.3. Using 5-nitroso-8-quinolinol, chelation occurred only with thallium, which precipitated at pH 5.3; 2-methyl-5-nitroso-8-quinolinol produced no chelates with any of these elements. Having in mind the unselectivity of 5-methyl-7-nitroso-8-quinolinol it would appear that here both acidity of reagent and steric hindrance encourage selectivity.

Dziomko (*35*) used organic reagents containing two quinolyl groups, and concluded that the precipitating ability of these reagents exceeded those containing only one group. Furthermore, the precipitates with the reagents containing two groups were more insoluble in various common organic solvents. The metals used in these experiments were aluminum, copper, cobalt, nickel, iron, and manganese. For the precipitation of a large number of metal cations, Alimarin and Kryukov (*8*) used 5,7-dibromo- and 5,7-diiodo-8-quinolinol. These reagents were specifically recommended for the precipitation of tetravalent vanadium. Charles and Freiser (*24*) made chelate stability measurements with *o*-hydroxyphenyl derivatives of 8-quinolinol, benzoxazole, and benzothiazole, and as a result, predicted that the thiazole derivative would be superior to the accepted oxazole compound in its selectivity for cadmium. The selectivity of the reagents increased in the following order: 8-quinolinol, 2-(*o*-hydroxyphenyl)-benzoxazole, 2-(hydroxyphenyl)-thiazole. The relative absence of selectivity of 2-(hydroxyphenyl)-benzothiazoline could not be explained satisfactorily. The specificity of some new organic reagents was discussed by Gillis (*44*). Diaminobenzidine gave a yellow precipitate with selenium (IV) but not with tellurium. Analogous reactions were given by various related aromatic diamino compounds.

PREPARATION OF PRECIPITATES

A brief but significant report on the effect of addition reagents on the physical characteristics of three distinct types of precipitates was published by Fischer and Rhinehammer (*39*). In the case of silver chloride none of the many reagents used influenced the crystal size, but some increased the flocculation rate. Although a few reagents hastened photolytic decomposition, Naphthol Yellow retarded this process. Further work on this effect could be useful. With nickel dimethylglyoxime none of the 42 addition reagents exerted any noticeable influence upon the physical characteristics of the precipitate. In the case of barium sulfate the major effect of the addition reagent was upon the size of the crystal;. new errors were not introduced and coprecipitation of impurities was not affected. The time of aging of the barium chloride solution containing addition reagent prior to its use as a precipitant of sulfate was significant, but when the addition reagent was added to the sulfate solution little or no effect was observed. With all precipitates the addition reagents had no effect on the accuracy of determinations. This is a fertile field of investigation and further work is needed.

HOMOGENEOUS PRECIPITATION

For the homogeneous precipitation of insoluble iodates Stine and Gordon (*92*) applied the hydrolysis of β-hydroxyethyl acetate to yield ethylene glycol for the reduction of periodate. This method avoids the usual gelatinous character of iodate precipitates which has been a deterrent in their wider applications. It was found that zirconium and iron(III) iodates could be precipitated by this technique. The authors used the method to advantage for the determination of thorium in monazite sands. With a single precipitation there was reduced contamination by foreign ions; with a double precipitation the method effected quantitative separation of thorium from large amounts of rare earths and phosphate.

Hagiwara (*48*, *49*, *50*) published a number of papers dealing with the separation of rare earth elements and associated metals. He discussed homogeneous precipitation by hydrolysis of urea and the effect on the rate of change of pH of various organic acids and ammonium chloride or sulfate (*48*). The pH at which initial precipitation of rare earth elements occurred was determined (*49*). Zirconium was quantitatively precipitated at pH 2.8, thorium at pH 5.5. Cerium was separated from lanthanum, neodymium, and samarium by hydrolysis in sodium hydroxide solution of pH 5.5 to 5.6. By slow precipitation in the presence of urea, ammonium chloride, and acetic acid, cerium was quantitatively recovered at pH 3.8 to 5.5. A promising method for the separation of cerium from rare earth elements was recorded by Willard and Yu (*103*). Cerium(III) was slowly oxidized in the presence of iodate to cerium(IV) by either bromate or peroxysulfate. Two precipitations with peroxysulfate gave the better separation from rare earth elements. The various factors which influenced the precipitation were studied in detail. This method produced a precipitate of varying composition which was readily filtered, washed, and ignited. For some unknown cause the results were slightly high, but this error was eliminated by treatment of the ignited oxide with strong sulfuric acid.

An investigation by Gordon and Firsching (*46*) of the homogeneous precipitation of barium chromate revealed that almost complete precipitation occurred at pH 5.5 to 6.0, and not at pH 4.6 as had been reported previously. Although the small amount of barium (0.1 to 0.2 mg.) which remained in the filtrate could be reduced by further decreasing the acidity, there was a simultaneous increase in the precipitation of strontium. About 40 mg. of strontium could be separated in a single precipitation from about 100 mg. of barium; equal amounts of the cations required a double precipitation. A procedure for the homogeneous precipitation of barium sulfate was described by Heyn and Schupak (*52*). The ammonium salt of (ethylenedinitrilo)-tetraacetic acid (ethylenediaminetetraacetic acid, EDTA) was used to complex barium, then barium sulfate was formed by increasing the acidity to pH 2 to 3 through the hydrolysis of ammonium peroxydisulfate at 60° C. While the data included cannot create enthusiasm for the method, the principle involved could be useful in other gravimetric methods.

Controlled cation release was also used for the determination of iron. MacNevin and Dunton (*63*) precipitated hydrated ferric oxide by gradual release of ferric ion from the EDTA complex. This was accomplished by the addition of hydrogen peroxide to an ammonium chloride solution of the iron complex. For the precipitation of well formed crystals of ammonium magnesium phosphate, Saint-Chamant and Vigier (*84*) controlled acidity through the slow liberation of ammonia by the reaction of ammonium chloride and aqueous methanolamine. Washizuka (*101*) precipitated copper sulfide from a solution containing thiourea, urea, and ammonium chloride. The precipitate obtained at pH 4.0 after 40 minutes of boiling was easily filtered and readily washed.

Homogeneous and heterogeneous precipitations of basic stannic sulfate from the viewpoint of coprecipitation of manganese(II) were compared by Gordon, Teicher, and Burtt (*47*). From a series of significant experiments they concluded that for micro amounts of manganese(II) there was essentially no difference in the amount of coprecipitation. For macro amounts of manganese(II) at higher temperatures (thus hydrolyzing the urea more rapidly), there was a relatively large amount of coprecipitation with the initial 25% fraction of tin precipitate. This was assumed to be due to a "supersaturated condition" and was largely eliminated by precipitation at 76° C. instead of 97° C. At 76° C. coprecipitation was not observed until about 50% of the tin had precipitated. After the quantitative precipitation of tin a large increase in the amount of coprecipitation occurred. The authors state that "adsorption following complete precipitation of the carrier must be considered an important factor in precipitation reactions," and that this factor contributes more to the final coprecipitation than does occlusion. In connection with studies such as these, it is possible that information on the characteristics of homogeneous precipitation could be obtained by application of the methods described by Johnson and O'Rourke (*59*). These authors dealt with the kinetics of heterogeneous precipitations which involve induction and growth periods. There would be interest in obtaining the relationships between coprecipitation and the induction and growth periods.

THERMOGRAVIMETRIC ANALYSIS

A significant contribution to thermogravimetry was recorded by Erdey, Paulik, and Paulik (*37*). In a preliminary note these authors record success in the incorporation of differential thermal analysis through the addition to the beam of a permanent magnet which moves in a coil. This improvement may bring about a better understanding of the nature of decomposition of precipitates than is obtained by the present thermograms. The details of this research, to be published soon, should be of considerable interest to the analytical chemist.

Unohara (*99*) continued his work on the application of thermal methods to mixed precipitates of the alkaline earth metals, giving results on the analysis of dolomite. Mixtures of calcium sulfate and magnesium sulfate were determined successfully by heating to 1100° C. to produce magnesium oxide (*100*). With a view to obtaining improved precision Spinedi (*90*) standardized certain experimental techniques for the thermoanalytical determination of calcium carbonate and the like in mixtures. Some methods of analysis have been rejected too readily on the grounds that the precipitate did not reveal "horizontals" in pyrolysis curves. The thiocyanate-benzidine method for copper, previously rejected for this reason, has been used successfully by Burriel and Goyanes (*19*), who merely added the alcoholic benzidine first and then the thiocyanate. The precipitate is stable up to 180° C. Similarly the rejection of zinc salicylaldoxime as a weighing form seems to have been an error. Rynasiewicz and Flagg (*83*) showed that direct weighing after drying at 110° C. could be done successfully; furthermore, the ignited precipitate yielded constant weight between 500° and 1000° C. rather than at 950° C., as indicated by previously published pyrolysis curves. Zagórski (*104*) described a simple inexpensive thermal balance and recorded a method for the electronic recording of the temperature-weight curve.

LIGHT ALLOY ELEMENTS

Lithium, Beryllium, Aluminum. To overcome errors such as solubility and contamination incident to the precipitation of lithium phosphate, Caley and Simmons (*22*) proposed a new procedure involving precipitation by choline phosphate in 50% 2-propanol. Potassium or sodium sulfates present no significant interference, although other metals interfere and must be removed. The optimum range of application was from 2 mg. to somewhat less than 250 mg. A useful technique was described to indicate the approximate lithium concentration with a view to determining the amount of reagent required.

Akiyama published a series of papers dealing with established methods for the determination of beryllium (*1–7*). One procedure (*1*) provided for separation from aluminum and iron by sodium hydrogen carbonate solution at 80° C. in a closed container. Beryllium was precipitated in the filtrate by 8-quinolinol. In another procedure (*2*) beryllium was separated from aluminum by alkali hydroxide. Other methods involved precipitation of beryllium by ammonia in the presence of borax (*3*), determination of tannin (*6*), and sulfate (*7*). Separation of sulfides of iron, cobalt, and nickel from beryllium was also discussed (*6*).

Majümdar and Sen (*66*) provided a useful method for the separation of aluminum from iron and chromium. In the presence of ferrous iron or chromium and an excess of α-picrolonic acid, aluminum hydroxide can be precipitated at pH 5.8 to 6.85 to remove iron, and at pH 6.25 to 7.05 to remove chromium.

RARE EARTH ELEMENTS

Thermolysis curves for yttrium hydroxide and oxalate were recorded by Duval (*32*). Conversion of the hydroxide to the oxide occurred at about 856° C., and of the oxalate at 680° C. There were no other weighing forms indicated.

NATURALLY RADIOACTIVE ELEMENTS

Thorium. A critical study of the large number of gravimetric reagents for thorium would be a desirable contribution. Each year a number of new reagents are reported, but little effort has been made to estimate their relative value.

Kall and Gordon (*60*) published useful data dealing with the precipitation of thorium oxalate. They describe the effects of acidity, variations in concentrations of oxalate, thorium, and ammonium salts, and time of digestion. The conclusion is reached that thorium oxalate is not so quantitatively insoluble as one would infer from the literature, and that "on the whole it would seem desirable to avoid, whenever possible, the use of an oxalate procedure for the determination of thorium." A useful contribution to the iodate method for thorium was published by Tillu and Athavale (*98*). Precipitations were made in 40% nitric acid containing sufficient oxalic acid to complex zirconium, titanium, and bismuth. Only in the presence of large proportions of these interfering elements was reprecipitation necessary. Thorium hydroxide was subsequently formed and converted to the oxide. The method was applied successfully to monazite sands salted with interfering elements. Crepaz and Marchesini (*28*) have recorded that *m*-nitrobenzoic acid precipitated thorium benzoate at pH 1.9 to 2.4. When pH was increased, basic salts of varying composition were formed, and at pH greater than 3.5 thorium hydroxide precipitated. Ammonium benzoate was the preferred precipitant. 2,4-Dichlorophenoxyacetic and guaiacyloxyacetic acids (*30*) have been used successfully for the determination of thorium in monazite sand. With the former precipitant direct weighing could be made after drying at 105° to 110° C. Sebacic acid was recorded by Rao and Rao (*81*) as a reagent capable of separating thorium from 70 times as much uranium. Double precipitations at pH 1.88 were required.

ALKALI AND ALKALINE EARTH ELEMENTS

Sodium, Potassium, Calcium, Strontium, Barium. Sources of small errors in the determination of alkali elements in silicates were discussed by Geilmann and Ganssle (*42*). The Lawrence-Smith method of decomposition produced losses of R_2O by volatilization, while the mixed acid procedure resulted in losses by spattering. Removal of ammonium salts by heating caused errors through decrepitation. Such salts should be treated with nitric and hydrochloric acids. Additional losses were due to adsorption or occlusion by such precipitates as barium sulfate, calcium oxalate or carbonate, magnesium-8-quinolinate, and the hydroxides of iron and aluminum. The use of porcelain vessels resulted in errors due to either base exchange or direct silicate formation. Platinum vessels were to be preferred.

Dupuis (*31*) used lithium cobaltinitrite to precipitate potassium hexanitritocobaltate(III) dihydrate at 40° to 55° C. The precipitate is hygroscopic but can be dried at 220° C. to obtain an accuracy within 0.7%. At 300° C. the weighed residue is $CoO + 3KNO_3$. Belcher and Robinson (*14*) precipitated potassium as the 12-phosphomolybdate, which was then converted to lead molybdate. The method is applicable within the range of 2 to 20 mg. and permits a fairly accurate and rapid determination of potassium. It is as selective but not so accurate as the cobaltinitrite method. Fluoboric acid was used by Manasevit (*68*) to precipitate potassium in the presence of lithium and sodium and in the absence of cesium, rubidium, calcium, barium, aluminum, and ammonium ions. The temperature of precipitation is 3° C., but the method is simple and inexpensive.

The precipitation of potassium by tetraphenylboron has been the subject of a number of good publications. There is agreement that precipitation at low acidities encourages interference from other cations and results in filtering difficulties. Although high acidities increase specificity, there is significant decomposition of the reagent even at room temperatures, resulting in contamination of the precipitate. Recognition of these facts has given rise to various new procedures. Geilmann and Gebauhr (*43*) have provided detailed information about optimum acidity, temperature for precipitation and drying, suitable type of washing liquids, etc. Sporek and Williams (*91*) recommended precipitation at 0° C. in solutions between 0.2*N* and 0.6*N* and obtained accurate results with amounts up to 25 mg. Cluley (*26*) used two methods to produce tractable precipitates and avoid decomposition of reagent. One method required precipitation at pH 6.5 in the presence of EDTA and thus avoided interference from some 15 cations. In the second method pH 2 was used, and, as in the first, precipitation was made at 20° C. Procedures for the determination of potassium in glasses and refractories were also recorded.

For the separation of manganese from calcium and magnesium in the analysis of cements, Ingram and Bean (*57*) preferred oxidation of the manganese by permanganate rather than by bromine. With permanganate negligible amounts of manganese were left in the calcium oxide and magnesium pyrophosphate precipitates, and relatively consistent results were obtained. However, the necessary evaporation may lengthen the procedure by 2 or 3 hours.

Penchev and Nonova (*73*) used picrolonic acid to precipitate strontium from neutral solution, then air dried the precipitate. It had the composition $Sr(C_{10}H_7N_4O_5)_2.8H_2O$.

An unexpected determinant of the crystal size of barium sulfate was noted by Fischer and Rhinehammer (*38*). The crystals were severalfold larger when the barium chloride solution had been allowed to stand at room temperature for at least 24 hours. These authors investigated the effects of sulfate and barium ion concentrations, excess barium chloride, acidity, temperature, and foreign ions. A procedure accurate to within 0.1% was proposed in which the reagents were added rapidly and little or no digestion period was required. However, coprecipitation of nitrate and ferric ion remains a serious problem. Schulek, Pungor, and Guba (*86*) used the electron microscope to study the influence of ammonium chloride on the precipitation of barium sulfate. It was confirmed that addition of this reagent at the time of precipitation improved the efficiency of filtration.

STEEL-FORMING ELEMENTS

Zirconium, Tantalum, Niobium, Chromium. Reagents for zirconium and associated metals are becoming numerous. Although little acceptable effort has been made to compare the efficiencies of the various reagents, some data are arising through practical applications of new methods. Klingenberg and others (*61*) determined zirconium in steel by precipitation by the mandelate, *p*-bromomandelate, and benzilate methods. Contrary to previous findings benzilic acid precipitations could be made in 10% hydrochloric acid solutions, thereby eliminating interference from iron and tin. Halomandelate, phosphate, and Alizarin Red S methods for the determination of the small concentrations of zirconium in magnesium alloys have been compared (*72*). The phosphate method was relatively time-consuming and the data indicate slightly low results. However, the reasons for the authors' preference for the halomandelate method were not made sufficiently clear. Mandelic acid and fluoro-substituted mandelic acid as reagents for zirconium were discussed by Belcher, Sykes, and Tatlow (*15*). These authors confirmed the general opinion that *p*-bromomandelic acid does not produce a precipitate which is a pure substance and that ignition is necessary. However, contrary to previously published data, even mandelic acid does not produce a precipitate of strictly constant composition, because it varies slightly with the amount of zirconium present. An empirical factor may be used, but good accuracy is obtained only when it is used for a narrow range of zirconium content or for small concentrations of zirconium. *o*-, *m*-, and *p*-fluoromethylmandelic acids were examined with a view to their use as precipitants. Apparently these reagents offer no significant advantages.

Bailey (*12*) described a method for the determination of tantalum and niobium based on the difference in stability of solutions of these pentoxides in ammonium oxalate solution. By carefully regulating the amount of tannin added to this medium the yellow tannin-tantalum pentoxide complex is precipitated in the presence of niobium.

Talipov and Antipov (*97*) separated trivalent chromium from sulfuric acid solutions of zinc, copper, nickel, cobalt, and cadmium by precipitation as the double salt $3NaF.CrF_3$.

Molybdenum, Tungsten, Nickel. Taimni and Agarwal (*95*) precipitated molybdenum(VI) sulfide dihydrate from a sulfide solution of molybdate by the addition of hydrochloric acid. The precipitate was purified by washing in a filtering crucible with water, ethyl alcohol, and ether and drying in a vacuum desiccator. Some useful researches on the analysis of titanium alloys have been recorded by Norwitz and Codell. In one of these (*70*) there are data concerning the determination of molybdenum as the sulfide. Hydrofluoric and sulfuric acids were used as the precipitating medium; hydrofluoric acid has the advantage of dissolving titanium alloys. Molybdenum was brought to the necessary valence of +6 by use of hydrogen peroxide, and a rapid stream of hydrogen sulfide was required for precipitation. Ignition to molybdenum(VI) oxide was accomplished at 500° C. The sulfide could not be weighed directly. The effects of associated metals were examined, and it was found that only tungsten interfered. This could be avoided by the addition of tartaric acid to the precipitating medium and to the wash liquid. Copper and tin also interfered. Cadmium nitrate was used by Jílek and Bieber (*58*) to determine molybdenum at pH 6 to 7. The precipitate was dried first at 110° C. and then at 270° C. Salts other than cadmium nitrate resulted in incomplete precipitation. Duval (*33*) had recommended cadmium molybdate as a weighing form for cadmium but not for molybdenum. The recommended drying range was 82° to 250° C.

For the separation of molybdenum and vanadium Malínek (*67*) used sodium sulfite to produce VO^{++} which was complexed by complexon II (EDTA). Molybdenum was subsequently precipitated by 8-quinolinol.

A rapid method of separating molybdenum and tungsten was proposed by de Sousa (*88, 89*). Calcium chloride was used to form calcium tungstate and calcium molybdate. The mixed precipitates were treated with concentrated hydrochloric acid to form tungstic acid and to dissolve the molybdate, which was then recovered from the filtrate as the calcium salt. The data indicate acceptable accuracy for both large and small amounts of the metals, and the analysis could be completed in one day

Improvements in the mercury(I)-tungstate method were discussed by Konopik (*62*). Ignition to tungstate(VI) oxide at 750° C. was accomplished in 5 to 10 minutes. This method requires very careful heating to avoid loss of tungstic oxide with the volatilizing mercury.

Byrn and Robertson (*21*) provided a useful method for the determination of nickel by dimethylglyoxime in the presence of iron and cobalt. The objectionable iron-cobalt complex was avoided by addition of *N,N*-dihydroxyethylglycine. Although a large excess of reagent caused chelation of nickel, this interference was removed by addition of excess zinc chloride. The results were accurate and no trouble was encountered in filtration.

NONFERROUS ELEMENTS

Copper, Zinc, Cadmium, Mercury, Thallium. A number of new reagents for copper have been recorded, but there appears to be little or no improvement over the many established methods. Byrn and Robertson (*20*) recorded the application to alloys of the useful reagent 2-(*o*-hydroxyphenyl)benzoxazole. Procedures for the determination of copper in nickel, in cast iron, and in aluminum are recorded. Ethylenediaminetetraacetic acid was used as a masking reagent and precipitates which formed with the latter reagent were removed prior to the addition of the benzoxazole.

For the precipitation of zinc by salicylaldoxime, Simonsen and Christopher (*87*) found that certain conditions for precipitation must be rigorously controlled. Digestion of the precipitate at 90° to 100° C. transformed the disalicylaldoximate into the mono salt, regardless of the acidity of the precipitating medium. These authors dried the precipitates at 80° C. and then ignited them to zinc oxide. Rynasiewicz and Flagg (*83*) stated that the correct drying temperature for this precipitate lay between 25° and 285° C., depending upon the moisture content and the drying rate. Drying to constant weight at 110° was satisfactory. Methylene blue, in the presence of excess thiocyanate ion, was used by Platunov and Mikhaĭlovskaya (*77*) to precipitate zinc. The complex was ignited to the oxide. Iron interfered but aluminum did not.

A useful method for the determination of cadmium in the presence of very large proportions of zinc was discussed by Rulfs and others (*82*). The precipitate of cadmium thiourea reineckate has a large gravimetric factor which is particularly attractive for microdeterminations. There is the usual interference from tin, antimony, copper, bismuth, mercury, and lead. The authors discussed the composition of the precipitate and the reasons for the good specificity.

Phenoxyacetic acid was used by Datta (*29*) to precipitate both mercury(I) and (II). At pH 3 there was no interference from lead or silver. The precipitate was heated to 100° C., dried in a vacuum, and weighed.

$NH_4Cr(CNS)_4(NH_3)_2.H_2O$ was used by Bagbanly and Mirzoeva (*11*) to precipitate thallium from $2N$ sulfuric acid solution. The crimson precipitate formed at 40° to 50° C. was dried at 110° C.

Germanium, Tin, Lead, Arsenic, Bismuth. Schrauzer (*85*) reported an interesting method for the precipitation of germanium by a reagent containing ammonium chloride, tartaric acid, and barium chloride. $Ba_2GeC_8H_8O_{14}.2H_2O$ was precipitated from an ammoniacal solution containing acetone, then washed and finally dried at 110° C. Arsenite did not interfere. The precipitation of germanium as tetraphenylarsonium-12-tungstogermanate was described by Phifer (*74*). Unfortunately, the method was not compared with the somewhat similar 8-quinolinol-germanomolybdate and 2-naphthoquinolinium-germanooxalate methods. Like the germanomolybdate method, the proposed procedure offers the advantage of direct weighing with the resulting favorable germanium factor. The impure oxalate complex requires ignition with the additional disadvantage that the decomposition products are injurious. It would be of some value to have these procedures compared from the viewpoint of accuracy, time required for determinations, and interferences. Phifer recorded interferences, some of which may operate but are not acknowledged in the existing similar precipitations. There is also the question of interferences caused by tetraphenylarsonium chloride as compared to precipitations as 8-quinolinates.

Taimni and Agarwal (*95*) precipitated tin(IV) sulfide dihydrate from a sulfide solution by addition of acetic acid. The yellow-brown precipitate was purified by organic solvents and dried in a vacuum desiccator. A method for the precipitation of lead after removal of tin and antimony was described by Hubicki, Frank, and Trau (*56*). At pH 4 to 5 orthophosphoric acid formed insoluble lead hydrogen phosphate which could be washed on hardened filter paper and ignited at 200° C. The method was accurate to within 0.13%.

Taimni and Agarwal (*94*) have recorded an interesting method for the determination of arsenic as the quinquevalent sulfide. The procedure involved conversion of the arsenic salt to a thio salt and subsequent precipitation of the arsenic sulfide. The latter may be purified in a filtering crucible by washing with water, alcohol, and ether and then drying in a vacuum for 30 minutes. Air drying at 100° to 110° C. may also be used. A thermogravimetric examination of this precipitate should be made.

A new precipitant for bismuth was reported by Burkhalter and Solarek (*18*). Trimethylphenylammonium iodide produced a precipitate which was easily filtered and could be oven dried to the tetrahydrate at 120° C. Precipitation was made from a cool 4*N* sulfuric acid solution from which halide ions were removed by fuming. There was no interference from associated metals. Although the noble metals and those of Group II interfere, these are readily separated from bismuth.

NOBLE ELEMENTS

Silver, Gold, Rhodium, Palladium, Osmium, Platinum. A promising method for the gravimetric determination of silver was reported by Cheng (*25*). In the presence of EDTA in ammoniacal solutions, 1,2,3-benzotriazole precipitated $Ag_2C_6H_4N_3$, which could be dried in 2 hours at 110° C. The compound was insensitive to light and practically insoluble in organic solvents and acetic acid, but was very soluble in concentrated mineral acids. It was also dissolved by cyanide and thiosulfate. There was no interference from such anions as chloride, bromide, fluoride, sulfate, nitrate, and phosphate. Iodide formed the lesser soluble silver iodide. Precipitations could be made in the presence of any metal ion. However, mercury(I) and iron(II) salts must be oxidized to their higher valencies. Ions which form insoluble hydroxides which are not complexed by EDTA—e.g., tin, antimony, titanium, and beryllium—required the addition of tartaric acid.

Harvey and Yoe (*51*) used the sodium salt of *N*-(*N*-bromo-*C*-tetradecylbetainyl)-*C*-tetradecylbetaine to precipitate gold. Two pure complexes could be obtained: one from hydrochloric acid solution, the other from hydrobromic acid solution. The method offers the advantage of a favorable weight factor, but filtration is difficult and the precipitating reagent is not commercially available.

Duval, Champ, and Fauconnier (*34*) found that both thionalide and mercapto-2-benzoxazole may be used for the direct determination of rhodium. The thionalide complex could be dried between 79° and 250° C.; the mercapto-2-benzoxazole complex between 92° and 150° C. Apparently no difficulties were encountered in washing out excess reagent. It is this problem which has precluded the direct weighing of various platinum metal precipitates.

Ayres and Berg (*10*) investigated the precipitation of palladium by dimethylglyoxime and concluded that the separation from platinum, iridium, and rhodium was nearly perfect. While the method is a good one, particularly for separation from both iridium and rhodium, it is by no means perfect under conditions which normally prevail in platinum metal analysis. deHovre (*55*) used *p*-aminoacetophenone in hydrobromic acid to precipitate palladium. Conventional methods of reduction were applied. *p*-Thiocyanoaniline was also recommended as a precipitant for palladium. Przheval'skiĭ, Shlenskaya, and Ogarkova (*80*) used this reagent at pH 0.25 to produce $PdCl_2.2C_5H_5NH_2$, which, although only stable to 150° C., could be dried at 130° to 140° C. There was no interference from divalent iron, copper, platinum, and iridium. The rather high acidity offers advantages in the precipitation of palladium. The efficiency of fire assay for palladium was investigated by Fraser and Beamish (*40*). The amounts of metal lost to the slag may be in the order of 1 to 3%. Losses of palladium during cupellation were negligible. An investigation by Gol'braĭkh (*45*) of the coprecipitation of copper during the formic acid reduction of platinum and palladium salts yielded some unexpected results. Precipitated palladium carried very little copper; in the case of platinum, if the copper content was less than the ratio of 1 to 1, practically all of the copper appeared with the platinum. Simultaneous precipitation of palladium and platinum in ratios of 4 to 1 or greater resulted in very little copper contamination.

Standard procedures for the determination of platinum, palladium, and gold in fire assay beads were discussed by Barefoot and Beamish (*13*). A new method for determining palladium in the sulfuric acid parting acid involved direct precipitation by sodium dimethylglyoxime.

The first quantitative organic precipitants for osmium were recorded by Hoffman and others (*53*). Procedures were described for gravimetric determination by thionalide, strychnine sulfate, and 2-phenylbenzothiazole. Ignition in hydrogen was accomplished using the catalytic action of osmium to produce the metal.

Champ, Fauconnier, and Duval (*23*) published the thermograms from seven methods used for the determination of platinum. Thiophenol was recommended for direct weighing. The precipitate could be dried between 230° and 300° C. This is suitable when the thermobalance is used, but will be much less applicable under conditions of general practice. The authors also found that ignited residues obtained from precipitations by magnesium and sulfide contained these elements as impurities.

NONMETALLIC ELEMENTS

Silicon, Selenium, Tellurium, Phosphorus, Fluorine. Armand and Berthoux (*9*) determined the optimum conditions for the precipitation of complex silicomolybdic yellow by quinoline. The influence of acidity, concentration of molybdate, temperature of precipitation, and the thermal properties of the precipitate were studied. The precipitate of $SiO_2.12MoO_2.4(C_9H_7N).2H_2O$ can be dried at 150° C. in 1 hour.

Taimni and Agarwal (*94*) published useful procedures for the quantitative precipitation of the quadrivalent sulfides of selenium and tellurium. Solutions of the selenite or tellurite were treated with sodium or ammonium sulfide followed by acidification with hydrochloric acid. The sulfides were purified by washing in a filtering crucible with water, heating at 100° to 110° C. in air, and cooling in a vacuum desiccator.

Precipitation reactions of condensed phosphates were studied by McCune and Arquette (*64*). The triphosphate $(H_2P_3O_{10})^{---}$ was precipitated almost quantitatively at pH 3.5 by tris(ethylenediamine)cobalt(III) chloride. About 93% of the pyrophosphate ion $(HP_2O_7)^{---}$ was removed at pH 6.5. Orthophosphate and tri- and tetrametaphosphates were not precipitated. The usefulness of the reagent was reduced by failure to effect a quantitative separation of the tri- and pyrophosphates. Some of the pyrophosphates were coprecipitated and some of the triphosphates remained in solution

An indirect method for the determination of fluorine was recorded by Popov and Knudson (*78*). Fluorine was distilled with perchloric acid and precipitated in a nitrate-acetate medium at pH 2.0 to 2.5 by an excess of standard lanthanum nitrate. The excess lanthanum was determined by cupferron.

VOLUMETRIC ANALYSIS

GENERAL

The most significant advances in volumetric analysis during the period under review have been brought about by the introduction of ethylenediaminetetraacetic acid. Indeed, this substance has in a space of five years advanced from the category of a special reagent to win a place equal to or greater than that of iodine in volumetric work. Procedures using EDTA are now available for the determination of 32 elements and a variety of radicals.

Various metal indicators and masking agents have been applied to improve the selectivity of the complexing principle. Clearly, further advances in the study of chelates will provide many more specific procedures. Among the masking agents which have been found useful in procedures involving EDTA are cyanide and fluoride, which can be used in obvious ways. Less familiar are the applications of triethanolamine and 2,3-dimercaptopropanol. For example, triethanolamine masks iron, aluminum, and manganese in nickel and calcium determinations (*88*). By combining the effects of triethanolamine and cyanide, procedures have been made available for determining magnesium, zinc, cadmium, and manganese in various mixtures (*89*). Lead, bismuth, zinc, cadmium, arsenic, antimony, and tin form soluble complexes in alkaline medium with 2,3-dimercaptopropanol. By taking advantage of this, calcium and manganese determinations may be carried out directly; lead, manganese, and zinc determinations can be carried out indirectly or after appropriate variations of the general procedure (*92*).

ter Haar and Bazen (*54*) point out that metal indicators applicable in strong acid media are not necessary if one back-titrates excess EDTA with thorium nitrate in the presence of Alizarin Red S, which shows a sharp color change.

Blaedel and Knight (*12*) showed, by means of high frequency titration with standard EDTA salt, that the complexing with several metal ions is stoichiometric. These authors have shown that EDTA can be purified for use as a primary standard (*13*).

A close analog to EDTA, 1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid was studied by Přibil (*91*). This reagent differs from EDTA in that it forms complexes with lead, zinc, cadmium, and nickel in acid solution. The fact that the complex with iron is stronger than the EDTA-iron complex should be of advantage.

Ascorbic acid is establishing its usefulness in analytical chemistry. Erdey (*29*) has used the reagent satisfactorily for determining a wide variety of oxidizing agents. Most of the attention to date has been directed toward a simple iron determination.

Because of the stability of its solutions, mercurous nitrate is superior to all other reducing titrants for iron. This reagent, in the presence of thiocyanate, was advanced some years ago by Bradbury and Edwards (*16*) for the determination of iron, and was recently examined by Belcher and West (*11*). The thiocyanate not only serves as an indicator, but is necessary for the reduction process as well. Burriel, Conde, and Jimeno (*20*) have applied mercurous nitrate to the determination of chromium(III), arsenic(III), hydrogen peroxide, and hydrazine.

The lack of a reversible indicator for bromate titrations is a great inconvenience By means of a spectrophotometer, Sweetser and Bricker accurately followed the course of these titrations for the determination of several substances (*115*).

Photometers are being used frequently to detect the change in color at the end point of titrations, such as those for calcium and magnesium (*67*). This technique often yields very accurate results, even though visual observation fails. Bobtelsky has used a photometer to detect the end of a variety of precipitation titrations, an example of which is the determination of calcium in the presence of magnesium (*14*). Although his method may sometimes be applied in cases where no indicator is available, its use will probably be limited, for it is neither accurate nor convenient.

Interest in high frequency end-point detection has continued, but much of the work has been directed toward new instrumental devices. These instruments are valuable when solutions are so highly colored or dilute that ordinary indicators fail. High frequency techniques can be used for complexometric titrations (*58*) and precipitation titrations such as those with oxine (*53*).

Although electrometric methods of titration are being reviewed elsewhere, the paper by Frisque and Meloche (*42*) may be mentioned here. It presents a mathematical treatment, supported by experimental evidence, which allows the analysis of mixtures of two weak acids. The method is to be applied in cases when a titration curve does not show two separate breaks. The results are calculated from the volume of titrant required to establish a calculated "minimum error" pH value. The results for two "medium weak" acids were good, with errors usually less than 5%.

Ballczo (*3*) has recommended the use of "double titration" when very dilute solutions are being used. This device is intended to provide an indicator error correction which is more valid than the usual one, but evidence of its superiority in this regard is not included

Apparent indicator constants for bromophenol blue, bromocresol green and phenol red were determined for ethanol-water and glycol-water solutions (*78*). In view of the increased use of mixed solvent systems, a growing interest in such data is anticipated.

DETERMINATION OF ELEMENTS

Aluminum. A procedure has been developed by Taylor (*119*) in which the sample solution is added to a hot solution of excess EDTA containing hematoxylin; the excess EDTA is then titrated with a standard aluminum solution. However, the results obtained were somewhat low. An accurate EDTA method has been developed by Theis (*120*), in which the principal known interferences are zirconium and zinc. This uses Chromazurol S as indicator. Iron, if present, is reduced with hydrazine sulfate. The method is direct and simpler than the oxine method, though possibly not so precise.

Antimony. Interference from copper in the iodometric determination of antimony(III) can be eliminated by complexing with citrate (*125*). The copper can be determined by acidifying and titrating with thiosulfate. Alternatively, Hamlin (*56*) determined antimony free from other reducible constituents by reducing with excess titanous chloride and heating the acid solution to oxidize the excess. The reduced antimony was then determined in conventional fashion.

Barium. For the determination of barium, Manns, Reschovsky, and Certa (*77*) titrated with EDTA and Eriochrome Black T, adding a little calcium and magnesium to the titrant to produce a color change. Sijderius (*107*) added the magnesium salt of EDTA to the sample and obtained better precision; but as he did not standardize the EDTA solution against barium, his results are higher than the theoretical.

Beryllium. Beryllium was determined indirectly by iodometric titration of the arsenate in a precipitate of beryllium ammonium arsenate (*1*). A more convenient method (*101*) makes use of the fluoride complex but is not so specific as the iodometric method.

Bismuth. Cheng (*22*) has improved, and examined for interferences in Schwarzenbach's EDTA procedure for bismuth. Fritz (*43*) has provided a further improvement. Of 28 metals added as contaminants, none interfered appreciably when appropriate masking agents were added. This procedure uses high acidity with a large excess of thiourea as an indicator.

Wennerstrand (*128*) separated bismuth from interfering elements in metallurgical products by precipitation with sodium diethyldithiocarbamate from a cyanide-tartrate medium, and then carried out an EDTA titration. The use of pyrocatechol violet in acid medium provides an end-point indication of good specificity.

Utsumi (*123*) applied an iodometric titration to a chromate precipitate of bismuth.

Boron. The interference in the determination of boron from elements occurring in borosilicate glass was eliminated by masking with EDTA (*93*). The customary glycerol or mannitol procedure can then be applied to the treated solution. The determination of boron is made more certain, according to Vizzi (*126*), by titrating to the change of bromophenol blue, adding mannitol, and continuing to the pink of phenolphthalein. A little *p*-nitrophenol may be added to mask the color of the first indicator.

Bromine. By using an ingenious procedure, Szábó and Csányi were able to oxidize bromide ion to bromate with chlorine water (*117*). They then titrated the bromate iodometrically Somewhat more accurate results were obtained by Schulek and others, using cyanide and chlorine water (*105*). The principle was applied also to the determination of hypobromite (*102*).

Cadmium. A method of high selectivity utilizes precipitation with 2-(*o*-hydroxyphenyl)benzoxazole (*127*) Unfortunately, volumetric determination of the organic ligand by a brominating procedure is not stoichiometric; however, it is reproducible to a fair degree. An apparently useful procedure, which uses diantipyryl-*o*-hydroxyphenylmethane, is that of Kumov (*71*). A precipitate is formed which may be weighed directly, or dissolved and titrated with base. By titrating with EDTA in a solution to which cyanide and formaldehyde have been added, cadmium may be determined in the presence of copper, nickel, cobalt, mercury, and small amounts of iron (*34*).

Calcium. The EDTA method was applied by Brunisholz and others (*19*) to samples from which phosphate had been removed by ion exchange. These authors report that a mixture of five parts of Eriochrome Black T to one part of methyl yellow is superior to murexide as indicator. Banewicz and Kenner (*4*) separated calcium from magnesium and determined both with EDTA. Flaschka and Jakoblewicz (*41*) determined calcium in the presence of magnesium by titrating an excess of EDTA with calcium chloride. These authors recommend a mixture of Naphthol Green B and murexide for the indicator. The procedure was applied to magnesite. In a study of the EDTA titration of calcium in the presence of magnesium, Bond and Tucker (*15*) estimated the influence of the amount of alkali added and recommended procedures to be used for water and soil samples. Sucrose or glycerol was employed to reduce errors.

Lime in slags was determined by high frequency oxalate titration after precipitating but not removing silica and hydroxides (*82*). This may be very convenient for rapid estimations.

Chlorine. Genchev made a comparison of the Mohr and Volhard methods with the gravimetric silver chloride determination (*49*), and stated the effects of various electrolytes. The Volhard method gave the most precise results when the amount of electrolyte present was varied, but there was a negative error. The interference of sulfate in the argentometric determination of chloride is counteracted, at least in part, by the addition of an equal volume of ethanol (*65*).

Considerable attention has been directed toward the determination of chloride with mercuric nitrate. The most significant contribution appears to be that of Parsons and Yoe (*85*), who used the sodium salt of diphenylcarbazidedisulfonic acid with ferric ion as indicator. The increased solubility of the sodium salt and its mercury complex over that of the simple diphenylcarbazone affords a much more distinct end point with a very small blank correction. An inert green solution used as a color screen is described. Iodate in alcoholic weak acid solution also provides a convenient and reasonably precise indicator by forming insoluble mercuric iodate at the end point (*30*).

Silver removes cyanide from the cyanide complex of nickel. Titration of the freed nickel with EDTA forms the basis of the determination of silver halide precipitates described by Flaschka and Huditz (*39*)

Aznárez and Vinadé extended their original procedure for the analysis of hypochlorite and chlorite mixtures to include chlorate by adding excess arsenite, acidifying strongly, and titrating with potassium bromate (*2*). Hypochlorite is titrated potentiometrically in this procedure. An iodometric procedure for chlorite in chlorine–oxy acid mixtures has been developed by Schulek and Endroi (*103*), using cyanide to convert hypochlorite to the inactive cyanogen chloride. Hypobromite and bromine cannot be inactivated in this way, however, because the bromine in cyanogen bromide possesses electropositive character and consequently is oxidizing in behavior.

Cobalt. An EDTA titration developed for cobalt by Harris and Sweet (*62*) was applied by the same authors to samples in which cobalt had been separated from nickel by means of 1-nitroso-2-naphthol precipitation, followed by a chloroform extraction of the cobalt complex (*61*).

Copper. As in the case of aluminum, Chromazurol S can serve as a complexometric titration indicator for copper (*121*). The titration carried out in weakly acid solution is influenced by only a few of the common elements, but the precision is not great. Catechol violet can be used as indicator (*112*), but an alkaline solution is required.

Fluorine. The excess of calcium added to a fluorite solution can be titrated with EDTA (*6*). Unfortunately, the calcium fluoride precipitate must be allowed to stand overnight and the filtration is troublesome. The direct titration of fluoride is to be preferred in most cases. Sommer (*110*) improved the thiocyanate end-point indicator for this titration by adding a layer of ether near the end of the titration; Miyahara recorded conditions for using salicylate as indicator (*80*).

Gallium. Accurate results for the determination of gallium were obtained by precipitating with 8-quinolinol, dissolving in hydrochloric acid, and titrating with iodate-iodide solution (*97*). A convenient method for determination consists of an EDTA titration at pH 2.8 with gallocyanine indicator (*79*). Results with this procedure are precise but somewhat high. The determination can be applied to samples after separating the gallium by ether extraction Both accuracy and speed are afforded by the method of Belcher, Nutten, and Stephen (*10*). The slightly acidic, alcohol-water solution is titrated with potassium ferrocyanide in the presence of either 3,3′-dimethylnaphthidine or its disulfonic acid.

Germanium. Germanic acid can be titrated with alkali after being complexed with fructose in a manner similar to the method using mannitol (*100*). It is not apparent if one substance is superior to the other.

Iodine. Gleu and Kattham have found that periodate in the presence of iodate may be titrated with arsenite by catalyzing the reduction with a ruthenium salt (*51*). Because this reaction proceeds in dilute acid solution, the procedure is more flexible than the usual iodometric method, which requires neutral or slightly alkaline conditions.

Indium. Titration with EDTA in boiling basic solution containing tartrate and Eriochrome Black T serves to determine indium (*37*). Cyanide makes the determination more specific. Patrovsky (*86*), using the fluorescence of the morin complex as an indicator, presented a procedure for the analysis of sphalerite. The titration in this case is carried out at room temperature.

Iron. Rothsche (*98*) studied the reduction of ferric iron with sulfur dioxide, zinc, or silver. Of these, silver was recommended. Chromate was preferred over permanganate or ceric sulfate for the oxidation. The contribution by Lang and Furstenau (*72*) is significant. These authors have shown that reduction of iron with thiosulfate allows a determination in the presence of a variety of elements including copper, arsenic, and antimony, when suitably modified procedures are followed. The errors due to the interference from copper in the dichromate titration of iron were overcome by masking with urea. Presumably, the masking could be applied when other oxidants are used.

White (*129*) regards the iodometric determination of iron as simpler than the dichromate or mercury methods. He recommends a hydrochloric acid concentration of 0.5 to 1.5N, a moderate excess of potassium iodide, and a volume of 100 ml. Two catalysts have been proposed for this determination: cuprous iodide in potassium iodide (*55*) and ammonium molybdate (*94*). Iron may be determined iodometrically in the presence of large quantities of sulfate without undue increase in the acid concentration if the cuprous iodide–potassium iodide catalyst is used (*57*).

Phosphate, even in large amounts, does not interfere in the titration of iron with the disodium salt of EDTA (*21*). The authors recommend a back-titration with ferric alum at pH 2.0 to 2.4, extracting the thiocyanate complex into amyl alcohol. It appears likely that copper would not interfere seriously at this acidity.

Promising procedures for the determination of iron by means of reducing agents are discussed in the general procedures given above.

Lead. The chlorofluoride method was studied by Belcher and Brewer (*5*). Results averaged almost 0.5 mg. low. More promising methods appear to be the EDTA titration modifications, which were described by Kinnunen and Wennerstrand (*70*) and Flaschka and Huditz (*40*) The former EDTA method is more specific involving an extraction of the diethyldithiocarbamate complex. The latter is direct and can be used in the presence of metals which form complex cyanides. Precipitation with thiourea as described by Mahr and Otterbein (*74*) is also useful.

Magnesium. The determination of magnesium by the difference of two EDTA titrations employing different indicators is convenient but is not precise for high calcium to magnesium ratios (*23*). Magnesium in limestone samples having calcium-magnesium ratios as high as 20 to 1 was determined with somewhat low results by titrating with EDTA after an ammoniacal oxalate precipitation (*4*). The precision of the titration appears to be good, and reprecipitation of the calcium may well allow an accurate magnesium determination.

Manganese. Manganese, in the divalent state, can be determined fairly specifically by using EDTA and Eriochrome Black T in a hot buffered cyanide solution (*32*). Increased specificity can be obtained by precipitation with trithiocarbonic acid (*87*) or thioacetamide (*35*).

An interesting iodometric method consists of air-oxidizing the complex formed between manganous ion and triethanolamine to form a compound which will release iodine from an iodide (*50*). Interference from a variety of ions can be prevented by adding Triton A.

Mercury. This element can be determined by first forming a mercuric oxide–acetone complex, adding potassium iodide or sodium thiosulfate, and titrating the liberated base with nitric, sulfuric, or perchloric acids (*24*). Accurate results are obtained in the presence of a wide variety of cations and anions including chloride.

Molybdenum. Gagliardi and Pilz (*47*) made a critical study of several methods of zinc reduction of the molybdate ion prior to a permanganate titration, and proposed a method giving very exact results. A somewhat simpler but less precise method using aluminum dust was advanced by Riegel and Schwartz (*96*).

Nickel. Two methods for nickel determination employing EDTA are of interest. The nickel in a dimethylglyoxime precipitate can be determined by dissolving the precipitate in hydrochloric acid, evaporating, adding excess ammonia, and titrating with EDTA using murexide as an indicator (*60*). This procedure possesses advantages over previously published volumetric methods applied to dimethylglyoxime precipitates. Cobalt is distinguished from nickel in the method of Přibil (*90*) by forming an EDTA–hydrogen peroxide complex with the cobalt and a cyanide complex with the nickel. Titration of the excess EDTA with magnesium salt gives the sum of the two metals; further titration after the addition of peroxide and cyanide gives the nickel content. It is to be expected that, in general, metals not reacting with cyanide would not interfere with the nickel result.

Niobium. Schwarz reduced niobium with zinc amalgam in the presence of tantalum, thus allowing its oxidimetric determination with ferric chloride (*106*). Zinc reduction of niobium was reported unsuccessful by Tomíček and Spurný (*122*), and these authors determined titanium in the presence of niobium by amalgam reduction. A clarification of this disagreement would be valuable, because the volumetric determination of niobium is of great interest.

Nitrogen. A new reagent, dicyclohexylthallic carbonate, can be employed in a volumetric determination of nitrate (*63*). In strong acid solution few anions interfere; among these, however, are the halides. Two volumetric procedures are described, one of which is very indirect. It involves precipitating the excess reagent with oxalate, filtering the sample, and titrating the filtrate. In the other more accurate modification, the thallium in the precipitate is determined.

Iron is oxidized by nitrate in strong acid solution, and Johnson modified an existing procedure using this principle (*66*). Accurate results may be obtained, but the manipulation is somewhat tedious. Similarly, Szábó and Bartha modified their existing procedure in which nitrate is reduced by ferrous iron in basic conditions (*116*). The reaction is catalyzed with silver diamine ion. Although the method can be recommended for its convenience, the results obtained were not precise.

High frequency apparatus was used by Tanaka and Nishigai for the titration of solutions containing as little as 2γ of ammonium hydroxide per ml. (*118*).

Palladium. Nickel displaced from the cyanide complex by palladium can be titrated with EDTA, using murexide as indicator. This procedure is applicable on a micro scale (*33*). Kinnunen and Merikanto report poor precision when murexide is used (*68*), and prefer back-titration of excess EDTA with manganous sulfate and Eriochrome Black T. Moreover, platinum does not interfere in this modification. The most accurate results for palladium using EDTA were obtained by MacNevin and Kriege by back-titration of excess reagent with zinc solution, detecting the end point with Eriochrome Black T (*73*).

Phosphorus. Red phosphorus can be determined by digesting it under reflux in ceric sulfate solution, then titrating the excess cerium with ferrous solution (*25*). Difficulties are encountered with white phosphorus.

Phosphate can be determined indirectly by EDTA titration of the magnesium in a precipitate of magnesium ammonium phosphate (*64*). Interference from a variety of metals was overcome by masking. An argentometric determination suitable for larger amounts than can be determined by the EDTA method uses pyramidone as indicator (*124*).

Potassium. Several papers have reported the use of sodium tetraphenylborate for the precipitation of potassium, and a number of volumetric modifications are now available. An argentometric titration may be carried out on the filtrate from the above precipitation as described by Rüdorff and Zannier (*99*). These authors eliminated interference from ammonium ion by the addition of formaldehyde. More specific procedures are those of Flaschka and others (*36*, *38*). The various methods using tetraphenylborate are roughly comparable in merit and a choice from them would depend on the particular needs of the analyst. The reader's attention should be drawn, however, to the discussion of potassium under the gravimetric section.

Selenium. Deshmukh and Sant developed an iodometric procedure for selenious acid in which the reduction is carried out in a chilled solution (*26*). An iodometric procedure for selenium, developed by Schulek and Koros (*104*), is free from the disadvantages of high acidity and consequent danger of iodine liberation. In this procedure the iodide is oxidized by cyanogen bromide, which is formed by treating the selenocyanide solution with bromine and then adding phenol. Errors are generally small.

Silver. Mannelli has proposed a determination of silver in the presence of lead to replace cupellation (*76*). Because the errors are large for lead-silver ratios greater than 7000 to 1, it is doubtful if the method will find much direct application for the purpose mentioned. However, a study in which application is made to partially cupelled buttons may discover a procedure in which errors are reduced.

An EDTA determination of silver chloride is mentioned under chlorine above.

Sulfur. Sulfide in blendes and ores was determined by distilling evolved hydrogen sulfide into a solution of zinc, adding iodine, and titrating the excess (*28*). Since the well known combustion method gives somewhat low results due to incomplete liberation of sulfur dioxide, it would be of interest to have comparative data.

Elemental sulfur in acetone extracts can be titrated directly near the boiling point with a standard cyanide solution and bromocresol purple indicator (*109*). The method is accurate and more convenient than some of the older methods.

Sulfuric acid can be titrated in nitric acid with sodium rhodizonate in the form of test paper (*81*). This device allows direct titration of acid solutions, whereas previous use of this reagent was indirect. Another direct titration is that of Fritz and Freeland (*45*) using barium solution and Alizarin Red S. This indicator, the authors state, provides a much more satisfactory end point then other substances used because it acts as an adsorption indicator. They admit, however, that accurate results can generally be expected only in solutions free from other salts, because coprecipitation errors are greater than in the gravimetric method.

The barium in either the precipitate or the filtrate from a precipitation of sulfate can be determined by adding excess EDTA and back-titrating with magnesium (*7*, *8*). Belcher, Kapel, and Nütten (*9*) obtained better results with 4,4′-diaminotolane than they did with benzidine when both were used in a similar way. The method seems to be superior to those using barium as precipitant.

Thorium. The only notable contribution to the volumetric determination of thorium is the EDTA titration. Fritz and Ford (*44*) applied this reagent and Alizarin Red S indicator to mesityl oxide extracts. Because the titration is carried out at low pH, many ions including uranyl do not interfere. Suk, Malát, and Ryba (*113*) used pyrocatechol violet for the indicator.

Tin. Two similar procedures (*52*, *95*) applicable to alloys, eliminate copper interference by precipitating copper by thiocyanate. The tin is reduced with phosphorus or hypophosphorus acid and reoxidized with iodine or iodate. Malaprade used antimony powder for the reduction (*75*). This may be more specific for reduction than stronger reducing agents; however, aluminum or iron still seems preferable because these metals can be completely dissolved or, in the case of the iron, removed with a magnet.

Tungsten. The EDTA titration of calcium in the solution resulting from hydrolysis of a calcium tungstate precipitate served as a determination of the anion (*111*). The author recorded the application of his method to tungsten minerals but, unfortunately, presented no data on either these or pure solutions.

Uranium. An extensive study by Sill and Peterson (*108*) of the reductor treatment in the determination of uranium has resulted in a much refined procedure capable of good accuracy in the milligram range. Bricker and Sweetser (*18*) determined uranium together with iron by means of a spectrophotometric titration with ceric sulfate. In order to avoid errors due to atmospheric oxidation catalyzed by iron, most of the anticipated titer must be added to the titration cell before the sample is introduced A novel procedure (*84*) has been proposed but not yet investigated thoroughly. It takes advantage of the catalytic effect of uranium on the photodecomposition of oxalic acid.

Hara and West (*59*) have examined the reaction between the trisodium salt of EDTA and the uranyl ion, using high frequency detection of the end point. The ratio of uranium to EDTA in the complex is 2 to 1, a proportion which is singular among those of the heavy metals. Although the complex is not very stable, the precision of duplicate titrations is good.

Vanadium. In seven publications dealing with the determination of this element, no apparent advantages over existing techniques are to be found.

Zinc. The well known use of ferrocyanide for zinc determination has been examined by Oka and Kanno (*83*). These authors include back-titration of excess ferrocyanide among their recommendations. Faller (*31*) determined zinc with EDTA after separation as sulfide. Kinnunen and Wennerstrand (*69*) have provided a selective procedure by a group of complexing steps and one extraction. A much simpler procedure is that of Flaschka (*39*). In this, a degree of specificity is attained by adding cyanide to the sample. Formaldehyde decomposes the cyanide complexes of zinc and cadmium, which are subsequently titrated with EDTA.

Zirconium. According to Dhar and Das Gupta (*27*), oxalohydroxamic acid gives with zirconium a quantitative precipitate of constant composition. The metal may be determined indirectly by hydrolyzing the precipitate with hydrochloric acid to form oxalic acid, adding excess titanous chloride, and back-titrating with ferric salt. Fritz and Fulda (*46*) have provided an accurate EDTA method using Alizarol Cyanone RC or Eriochrome Cyanine RC at pH 1.3 to 1.5. Of a large number of metals, only bismuth, molybdenum, antimony, thorium, and tungsten interfere in all oxidation states with both of two procedures developed.

MULTICOMPONENT SYSTEMS

Direct EDTA titration methods for calcium and magnesium in limestone and biological materials have been presented by Gehrke, Affsprung, and Lee (*48*). A rapid separation of the calcium is effected with sodium sulfite. This direct magnesium titration is more rapid and the calcium procedure is much more precise than the older 8-quinolinol and the oxalate methods, respectively.

Both copper and iron in alloys were determined accurately by Brasted (*17*). Two aliquots of solution were titrated, with and without the addition of fluoride, and the iron content was obtained from the difference. The same author recommends the addition of sulfamic acid in copper determinations so that the titration may be carried out in nitric acid solution. For rapid routine work, the consequent elimination of the evaporation and neutralization steps is clearly advantageous.

Suzuki (*114*) has integrated a group of volumetric procedures to form a scheme of analysis for mixtures of iron, arsenic, antimony, and tin. Sweetser and Bricker (*115*) made use of the absorbance of the antimony(III)-chloro complex for its determination by a spectrophotometric method. Arsenic may be determined at the same time.

Yamatera (*130*) utilized the difference in stabilities of the alkali triiodides to effect a determination of rubidium and cesium in alkali mixtures. Iodine is liberated from the two triiodides by extraction with carbon tetrachloride. It is extracted only from cesium triiodide, however, when a carbon tetrachloride solution containing 0.00075 gram-atom of iodine per liter is used. It is probably most important to maintain the proper conditions rigidly; in view of the dangers of unwanted decomposition and occlusion, the procedure may be unreliable. It possesses the advantage of being more rapid than gravimetric methods.

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Volumetric and Gravimetric Analytical Methods for Organic Compounds

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THE analytical methods described in the following pages are selected from the literature which has become available to the reviewers from October 1953 to November 1955. An effort has been made to present only methods that show a reasonable indication of being of real value. The ultimate test of the value of the methods described here will be their usefulness in future applications.

In covering the literature of recent years it will be observed that the use of ion exchange resins has been, and continues to be, an important adjunct to analytical chemistry.

The titration of cationic detergents with anionic detergents (and the reverse), in which the indicator is equally distributed between an organic and aqueous phase at the end point, continues to receive study and application.

The determination of various organic compounds by oxidation with ceric solutions also continues to be investigated.

DETERMINATION OF ELEMENTS

CARBON AND HYDROGEN

The total carbon of several organic compounds in aqueous solution was determined with an accuracy within ± 4% by treatment of the acidified solution with potassium persulfate in the presence of silver nitrate. The liberated carbon dioxide is passed through an upright condenser and absorbed in weighed Ascarite tubes. A second treatment is necessary and precautions must be taken to prevent absorption of carbon dioxide from the air (*50*).

The product obtained by the thermal decomposition of silver permanganate has several unusual properties which make it useful as a combustion catalyst in the determination of carbon and hydrogen in organic compounds. The combustion is carried out at 450° C. and requires 1 hour for a semimicroanalysis. The advantages of the new filling are claimed to be the easy standardization of the catalyst, which may be used without previous testing, and the long catalyst life of 100 analyses. A deviation of ± 50° from the 450° combustion temperature still gives good results. The catalyst also absorbs sulfur dioxide and the halogens (*79*).

Tritiated organic compounds may be analyzed by combustion in oxygen, absorption of the resulting water, and determination of the tritium with a Geiger counter. The method gives sufficiently accurate results to make tritium appear useful as a tracer element. The method may also be used for the determination of carbon-14 (*161*).

HALOGENS

Sodium or lithium diphenyl in ethylene glycol dimethyl ether is used to decompose both aliphatic and aromatic halides. Following extraction with water, the halides can be determined by ordinary procedures. The reagent can be used after 1 year when stored at 5° in bottles with foil-lined caps (*83*).

Mikl and Pech (*93*) modified their method for determination of halogens and sulfur by impregnating filter paper with a solution of the compound instead of wrapping the compound in the paper before burning in a bottle of oxygen.

A similar method is reported for the microdetermination of halogens (*125*).

Ethylene dibromide and ethylene chlorobromide in air may be absorbed in ethyl alcohol and decomposed with sodium hydroxide, and the liberated halide determined by the Volhard titration (*72*).

Chromous ion will react quantitatively with carbon tetrachloride and chloroform by replacing all the chlorine with hydrogen. After the reaction, the excess chromous ion is titrated with ferric alum using thiocyanate as the indicator (*74*). The chromous ion reagent is prepared by reduction of potassium dichromate in a liquid zinc amalgam reductor.

A method for the combustion of organic compounds containing fluorine is carried out with oxygen in a quartz tube with auxiliary external heating, followed by a secondary combustion zone utilizing gas or hydrogen with excess oxygen. The gas is passed at a very high velocity, which practically eliminates corrosion of the quartz. The fluorine is converted to hydrogen fluoride, which is absorbed quantitatively in 1N sodium hydroxide (*165*).

Belcher (*13*) has found that a bed of sodium fluoride heated to 270° is more satisfactory than lead chromate for the absorption of silicon tetrafluoride. After recovery of the fluorine, the gravimetric determination as PbClF is more exact than the titration with thorium nitrate. A procedure is also suggested for determining the four halogens in a single sample.

A method for the rapid determination of silicon and halogens is described (*62*), wherein the sample is fused with a mixture of ethylene glycol and sodium peroxide in a metal bomb. The fusion product is dissolved in water, boiled to remove hydrogen peroxide, acidified with nitric acid, and then neutralized with ammonia solution. An ammoniacal suspension of zinc oxide is added and the precipitate is filtered and ignited. The silica in the residue is determined by the usual procedures. Chlorine, bromine, and iodine are determined by conventional methods; the fluorine is determined by precipitation as calcium fluoride or by titration with zirconium tetrachloride.

An automatic combustion apparatus for the determination of sulfur and halogen is described in which the sample is vaporized in a stream of nitrogen, and oxygen is injected in a high temperature zone for combustion. A thermocouple senses the heat generated by the combustion and controls the amount of heat for vaporization. An operator can complete 15 to 25 analyses in an 8-hour day with virtually no possibility of losing an analysis from improper combustion (*164*).

METALS

A simple, rapid titrimetric method for determination of tetraethyllead is described by Milner and Shipman (*94*). After the extraction of the lead by hydrochloric acid in the usual way, the lead is titrated at pH 10 in ammoniacal tartrate solution with disodium ethylenediamine tetraacetate [disodium(ethylenedinitrilo)tetraacetate].

NITROGEN

The Kjeldahl method continues to be the object of a great amount of careful research, which seems to emphasize the importance of the method for the determination of nitrogen.

Bradstreet (*21*) has published an excellent review of the Kjeldahl method from 1939 to 1953 to supplement his previous review. The review discusses the digestion, catalysts, distillation, and determination of ammonia, as well as application to more complicated forms of nitrogen.

Bradstreet (*22*) also presents a study of the determination of nitro nitrogen by the Kjeldahl method. Increasing the digestion temperature by using 18 instead of 10 grams of potassium sulfate gave the best results. A study of various hydroxy compounds which help in the conversion of nitro groups showed that 1-naphthol–pyrogallol (1 to 1 mixture) gave the best results and was superior to salicylic or thiosalicylic acid.

In a joint study by 27 collaborating laboratories on fertilizer, feed, and *S*-benzylthiouronium chloride samples, it was found that mercury is superior to copper as a digestion catalyst and that standard acid is preferable to boric acid for the absorption of ammonia. Certain sections of the AOAC "Official Methods of Analysis" (7th edition) have been revised as a result of the study (*12*).

In a series of papers on the Kjeldahl method, Takeda and Senda (*154*) report the results of a study of digestion conditions in which a mixture of selenium, mercury oxide, and copper sulfate with sulfuric acid and potassium sulfate catalyzed effectively the digestion of amino compounds but gave low results with nitro compounds. The addition of glucose in the absence of mercury oxide was effective for nitro compounds except volatile stable substances such as 2,5-dichloro-1-nitrobenzene. A pretreatment of nitro compounds with potassium hydroxide and ethyl alcohol appeared to improve the analysis of mononitro compounds but failed for di- and trinitro compounds.

A method is described by Datta (*33*) for determining nitrogen in protein and nonvolatile nitro compounds by heating a finely divided sample with a large excess of a 3 to 1 calcium oxide–sulfur mixture. The nitrogen is reduced to ammonia, which is determined in the usual way by absorption in acid.

OXYGEN

A modified Unterzaucher procedure for determining combined oxygen in sulfur-containing compounds uses a roll of copper gauze at 900° to remove sulfur as recommended by Oita and Conway (*101*). A bed of equal parts of carbon and platinum converts oxygen to carbon monoxide at 900° instead of 1200°. The fillings may all be in a tube in the same furnace (*102*).

SULFUR

In a variation of the usual methods, the sulfate solution obtained by nitric acid oxidation of the sample is treated with barium chromate. After the barium sulfate is removed by filtration, the excess chromate in the filtrate is titrated with $0.05N$ ferrous solution (*156*).

When tri- and tetramethylenethiourea are oxidized by nitric acid in an open vessel, sulfur values are too low because volatile carbon oxysulfide is formed. If the fumes from the oxidation are passed through a combustion tube over platinum catalyst at 500° the sulfur from the carbon oxysulfide is oxidized to sulfur trioxide, absorbed, and determined as sulfate (*144*).

A method for the determination of sulfur in some plant and animal material is described, in which the sample is dissolved in a solution of sodium hydroxide, evaporated to dryness, and heated gradually with the addition of potassium chlorate. The resulting sulfate is determined gravimetrically (*147*).

Sulfur in organic compounds and fuel is determined by burning the sample in a stream of oxygen using amorphous chromic oxide as a catalyst (*38*).

An improvement in a quartz tube combustion method for small amounts of sulfur is reported. Ceria on alumina when used as a catalyst is not affected by chlorine. The sulfate formed in the absorbers is precipitated with a known amount of barium chloride and the excess barium ion is titrated with disodium ethylenediamine tetraacetate (*166*).

A semimicromethod for the determination of sulfur is based on the hydrogenation of the compound in a porcelain tube in the presence of a platinum spiral heated to 850° by an electric furnace. The liberated hydrogen sulfide is absorbed in a buffered zinc sulfate solution, an excess of $0.02N$ iodine is added, and the excess is determined by titration with $0.02N$ sodium thiosulfate. Compounds containing phosphorus poison the catalyst (*170*).

Sulfur is determined in coals, coke, and organic substances by reduction with zinc vapor. The sulfide is liberated as hydrogen sulfide with hydrochloric acid, and determined by titration with iodine after absorption in a solution of cadmium acetate (*80*).

FUNCTIONAL GROUPS

ACIDS

Anhydrous pyridine has been used as the solvent in the titration of formic and benzoic acids (*158*). The titrants, piperidine, ammonia, and diethanolamine, were employed with a bromothymol blue indicator.

An improved method of determining citric acid (*51*) involves its precipitation as the lead salt, conversion of the salt to lead chromate, and the iodometric determination of the chromic acid produced upon treatment with hydrochloric acid. Interfering proteins can be removed with trichloroacetic acid or phosphotungstic acid.

Oxidative schemes for the analysis of acids have been limited to specific determinations. Thus, lactic acid is oxidized by ceric sulfate (*85*) to acetaldehyde, which is absorbed in buffered sodium bisulfite, the excess bisulfite being estimated iodometrically. The acceleration of the lead tetraacetate oxidation of formic acid (*105*) to carbon dioxide by potassium acetate renders the reaction suitable to a manometric method.

The conversion of salts of organic acids to the free acid by ion exchange resins (*160*), followed by neutralization, permits the determination of salts. In the application of such a procedure to calcium gluconate (*163*), it was found that stabilizers present in the sample affected the results or else rendered the method useless.

Acetates of calcium, magnesium, zinc, manganese, lead, and mercury (*31*) can be determined by using nonaqueous media. The salts are titrated in mixtures of ethylene or propylene glycol and a higher molecular weight alcohol, hydrocarbon, or chlorinated hydrocarbon, using perchloric or hydrochloric acid and a thymol blue indicator.

ACID ANHYDRIDES

The method of Hogsett, Kacy, and Johnson (*60*) for the determination of chrysanthemum acid anhydride in commercial allethrin has been extended to seven other anhydrides (*65*). Results are precise and accurate over a wide range of concentrations; most anhydrides react within 5 minutes.

The titration of *N*-carboxy-α-amino acid anhydrides (*16*) with sodium methoxide and a thymol blue indicator can be accomplished to within 2% of the equivalence point using the following solvents: methanol, benzene-methanol, acetophenone, dimethylformamide, pyridine, aniline, butylamine, acetone, ether, dioxane, chloroform, and ethyl acetate.

ALCOHOLS

The conversion of water-soluble alcohols to the insoluble nickel xanthate salt has been used as a basis for analysis (*4*). The nickel in the salt is determined by titration with ethylenediaminetetraacetic acid.

The use of propionic anhydride as a reagent for the estimation of hydroxyl groups in primary, secondary, and tertiary alcohols, polyols, and sterols has been described by Pesez (*106*). The method appears to have some advantage over acetyl numbers, since the propionyl numbers of certain tertiary alcohols are correct to within 3% of the calculated value.

Oxidation procedures for both ethyl alcohol (*7*) and isopropyl alcohol (*43*) have been described. The ethyl alcohol is oxidized to the aldehyde with a potassium dichromate–potassium sulfate reagent, the excess being determined iodometrically; the isopropyl alcohol is oxidized to acetone, which is absorbed in excess hypoiodite. The excess iodine is titrated and the amount of acetone calculated.

An alkaline solution of potassium cyanide has been employed by de Jong (*68*) in the determination of methylol groups in the condensates of urea and formaldehyde. The excess cyanide is titrated with mercuric nitrate. The method is superior to the sodium hypoiodite procedure when the polymer contains easily oxidizable groups.

Similar procedures have been reported for the periodic acid determination of glycerol, the principal variation being the method used to estimate or destroy the excess perioate. In one method (*114*), the excess periodic acid is treated with potassium iodide, followed by titration of the liberated iodine with sodium thiosulfate, while in another (*37*), the excess periodate is destroyed by reaction with ethylene glycol. Hartman (*58*) indicates that the reaction time for the oxidation of glycerol is less than previously believed, and recommends the use of potassium dimesoperiodate, $K_4I_2O_9$, for the oxidation of large samples of glycerol because of its greater solubility in water.

ALDEHYDES AND KETONES

A modification of the argentometric procedures of Ponndorf (*109*) has been found useful for the determination of pure aldehydes and mixtures of aldehydes and ketones (*132*). An accuracy within ± 2% is obtained with pure aldehydes. The main disadvantages of the procedure are the difficulty in detection of the end point and interference by cyclohexanone, cyclopentanone, and polyhydroxy alcohols.

The hydroxylamine hydrochloride procedure has been extended and adapted for the determination of carbonyl groups in oxycellulose (*142*) and of high molecular weight aldehydes and ketones in the presence of free carboxylic acids (*91*). Johnston (*67*) describes a modification of the procedure which avoids acetal formation and involves the use of *tert*-butyl alcohol. The procedure is recommended for aldehydes and ketones which react at room temperature.

The cyanohydrin reaction has been extended to the determination of acetaldehyde and propionaldehyde (*148*).

The determination of aldehydes by the bisulfite method followed by iodometric estimation of excess bisulfite has been evaluated (*86*). The most important modification in the procedure involves the use of a phosphate buffer in the aldehyde-bisulfite reaction (*119, 120*). Applications of the method to compounds such as metaldehyde (*126*) and various mixtures such as acetaldehyde in vinyl acetate (*70*) and acetaldehyde in the presence of crotonaldehyde (*136*) have been successful.

The reaction of phenylhydrazine or substituted phenylhydrazines with aldehydes and ketones is the basis of several methods of analysis. The following titrimetric procedure has been described (*157*).

To a measured volume (8 to 15 ml.) of a 2.5% solution of phenylhydrazine hydrochloride in 3:5 aqueous pyridine, add a 0.05- to 0.16-gram sample, stopper the flask, allow to stand several hours in the dark (a blank is run simultaneously), and titrate the excess phenylhydrazine rapidly with 0.1 to 0.2*N* cupric acetate, after addition of 5 to 7 ml. of ether. The end point is the color change from yellow-brown to brown-green. The cupric solution is standardized iodometrically.

Meyer (*92*) recommends the use of Orange-G or Chromazon Red A as indicators in the titration of aldehydes or ketones in the presence of sodium acetate with phenylhydrazine hydrochloride. Orange-G appears to be the better indicator, as the color change is more easily recognized.

Another similar procedure (*169*) involves the use of 2,4-dinitrophenylhydrazine in hydrochloric acid: The amount of hydrazine is determined before and after reaction by azotometric methods.

A gravimetric procedure for the determination of the carbonyl group has been applied to allethrin (*55*). The 2,4-dinitrophenylhydrazone is separated from the other components by chromatography and weighed.

The condensation of aldehydes with compounds containing active hydrogens has been applied to the gravimetric determination of aldehydes and ketones.

Spencer and Henshall (*139*) present kinetic data for the dimedone-formaldehyde reaction, which enable calculation of optimum conditions for the analysis.

Cyclohexanone can be determined by condensation with piperonal, anisaldehyde (*26*), or furfural (*25*).

Other ketones to which the method has been applied using anisaldehyde include *tert*-butyldimethylacetophenone and 1,3-dimethyl-2-acetyl-4,6-dinitro-5-*tert*-butylbenzene (*18*).

Both α- and β-diketones may be determined by reaction with bromine; the excess is determined idometrically (*95*). The authors discuss the effect of mercuric sulfate as a catalyst, the nature of the reactions consuming the bromine, and the behavior of several ketones under these conditions.

Derivatives of the aldehydes and ketones may be analyzed by application of an appropriate procedure.

Acetals, after hydrolysis to the aldehyde with 2*N* sulfuric acid, are estimated by the method of Siegel and Weiss (*132*).

Schiff bases can be titrated quantitatively in glacial acetic acid with perchloric acid, using methyl violet as the indicator (*42*).

Sant (*121*) reports that a method employed for the analysis of alkaline solutions of hydroxylamine may be useful in the determination of oximes. Hydroxylamine is oxidized to nitrogen with ferricyanide and the ferrocyanide produced is titrated with ceric sulfate.

Oximes and semicarbazones can be determined gravimetrically (*98*) after an exchange reaction with 2,4-dinitrophenylhydrazine.

AMIDES

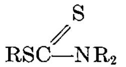
The reduction of amides and substituted amides to amines by lithium aluminum hydride

$$2RCONR_2 + LiAlH_4 \rightarrow 2RCH_2NR_2 + LiAlO_2$$

is the basis of a method of amide determination (*135*). Steam distillation of the amine from the reaction mixture is followed by titration with standard acid.

The hydrolysis of ethyl- and isopropylphenylcarbamates by acid or base to form aniline can be utilized in the analysis of these compounds (*11*). The aniline is estimated by titration with standard sodium nitrite solution, using a starch indicator.

Thioamides can be determined by the sodium hypoiodite titration method (*168*) or by the peroxide oxidation method of Kitamura (*75*). Bromometric methods fail. Methods of analysis applicable to derivatives of dithiocarbamic acid,



$$RS\overset{\overset{S}{\|}}{C}\text{—}NR_2$$

I

depend largely upon their structures. Compounds with secondary nitrogen can be estimated by the sodium hypoiodite method, bromometrically, or gravimetrically by oxidation with nitric acid or alkaline hydrogen peroxide followed by precipitation of the sulfate formed. Compounds with tertiary nitrogen require more vigorous oxidation procedures.

AMINES

Several of the procedures available for the determination of amines involve a perchloric acid titration of the free amine or its derivatives in nonaqueous media.

In one such method (*159*) malachite green, gentian violet, saframine, and orange I were found to be useful indicators in a 98 to 98.8% formic acid solvent.

Owens and Maute (*103*) recommend the use of acrylonitrile as a solvent for the determination of strong organic bases using a bromothymol blue indicator. Weak organic bases are best titrated using a 1 to 1 acrylonitrile–acetic acid solvent and crystal violet as the indicator. Both procedures are rapid and reliable; neither requires a blank correction.

Aminophenols and various alkaloids (*32*) may be titrated in ethylene or propylene glycol and a higher alcohol, hydrocarbon, or chlorinated hydrocarbon solvent mixture using a thymol blue indicator.

Perchloric acid in an acetic acid–acetic anhydride mixture can be used to titrate amine picrates (*28*) to an accuracy within 1%. A glacial acetic acid solvent is used along with a methyl violet indicator.

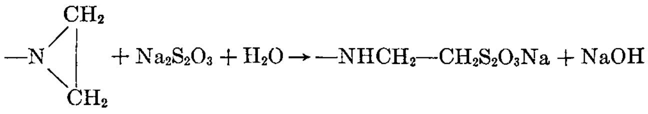
Complex formation between tellurium tetrabromide and organic nitrogenous bases is the basis of a titrimetric procedure for amine determination (*36*). Compounds such as diethylamine, aniline, pyridine, and piperidine were determined to within 1% of the equivalence point in glacial acetic acid, using concentrated alcoholic solutions of cupric chloride or cobalt bromide as indicators. Fatty acids and alcohols do not interfere.

Solutions of aromatic amines, such as aniline, *o*- and *p*-aminophenol, and 1- and 2-naphthylamine were analyzed using an excess of ceric sulfate (*153*), with a maximum error of 2%. The excess ceric sulfate was determined by back-titration with ferrous sulfate.

Total amino value and partial amino value have been defined and applied to the analysis of amine mixtures of high molecular weight (*118*). The total amino value is the number of milligrams of hydrochloric acid required to neutralize 1 gram of amine sample, while the partial amino value is the number of milligrams required to neutralize secondary and tertiary amines in the sample. Standard solutions of hydrochloric acid in alcohol are employed.

Secondary amines can be separated from tertiary and primary amines by forming the nickel dithiocarbamates and extracting the primary and secondary derivatives from the mixture with a sodium hydroxide–ammonium hydroxide solution (*99*). The nickel in the secondary amine dithiocarbamate can be titrated with ethylenediaminetetraacetic acid (murexide indicator) after displacing the nickel with silver ion in the case of small samples or decomposing the nickel dithiocarbamate with nitric acid in the case of larger samples.

The rapid reaction of ethyleneimine derivatives with excess sodium thiosulfate at pH 4



$$-N\begin{cases}CH_2\\ CH_2\end{cases} + Na_2S_2O_3 + H_2O \rightarrow -NHCH_2-CH_2S_2O_3Na + NaOH$$

consumes 1 mole of acid for each imino group present (*2*) and therefore may be used to estimate this type of compound. The acid consumed is measured by back-titration of the excess standard acid with standard alkali. The average deviation in the method is ± 0.1%.

Experiments show that in the determination of methyl- and ethylimide groups, errors are introduced as a result of reaction of the alkyl iodide produced with sodium thiosulfate used in the analysis (*41*). The authors recommend that a 10% solution of sodium antimonyl tartrate, a 5% alkaline solution of hydroxylamine hydrochloride, a 5% ascorbic acid solution, or a 5% hydrazine solution be used in place of sodium thiosulfate.

Gravimetric procedures have been applied to the determination of individual compounds.

Triethanolamine hydrochloride can be determined with an accuracy within 2 to 7% using sodium tetraphenylboron as a precipitant at a pH of 5 to 6 (*100*). Application of the method to mixtures of potassium chloride and triethanolamine hydrochloride gives results which are about 2% low in both components.

Piperazine in dilute aqueous solutions is precipitated quantitatively with excess ammonium molybdate as $3C_4H_{10}N_2.10MoO_3.$-$8H_2O$ (*27*). The compound is dried at 100° and weighed.

Pyridine has been estimated with accuracy within 1 to 3% by adding copper nitrate and standard potassium thiocyanate to precipitate $Cu(C_5H_5N)_2(SCN)_2$ and then determining the excess thiocyanate (*97*).

AMINO ACIDS

After reviewing 55 papers dealing with the determination of serine and threonine, Schlormüller and Walter (*127*) conclude that the two most important methods involve oxidation with lead tetraacetate or with periodic acid. Both procedures give accurate results, but the periodate procedure is to be preferred.

The following procedure has been suggested for the determination of glycine, alanine, arginine, and cystine (*151*).

Mix 5 to 25 ml. of a 0.1% aqueous solution of the amino acid with 2 ml. of a 30% sodium nitrite solution and 20 ml. of 3*N* sulfuric acid, and dilute to 70 ml. After heating for 15 minutes, add 50 ml. of 0.1*N* ceric sulfate solution, heat again for 15 minutes, cool, and titrate the excess ceric ion with 0.1*N* ferrous sulfate. Urea interferes with the determination.

An investigation of the action of periodic acid on amino acids and *N*-methylamino acids (*82*) indicates that the method is not applicable to a quantitative determination of these compounds.

ENOLS

The use of methanolic iodine monochloride in the Kurt Meyer enol titration results in increased accuracy and rapidity (*48*, *49*). Solid sodium bicarbonate, which is insoluble in the methanol solvent, is used to remove hydrochloric acid as it is formed, thus eliminating the possibility of producing enol by acid catalysis.

Bokadia (*19*) has made a critical study of the Meyer enol titration when applied to the determination of enol in 2-formyl ketones, 2-formyl bromoketones, and certain unsaturated ketones. Errors and corrections which arise in the determination of these compounds are discussed.

ESTERS

Aminolysis of phenolic esters with ethylenediamine permits application of the Fritz and Keen (*44*) phenol procedure to the analysis of these substances (*52*).

A determination of benzoyl groups (*123*) involves hydrolysis with 9*N* sulfuric acid or 5*N* sodium hydroxide, and extraction of the benzoic acid produced with chloroform, followed by titration with sodium methoxide to a thymol blue end point.

Ethyl ethylmalonate as a contaminant in ethyl diethylmalonate samples can be determined as follows (*56*) when present in concentrations greater than 1%.

Adjust 3 ml. of the sample, in a glass-stoppered test tube, to 20° C., add 10 ml. of anhydrous *n*-propyl alcoholic potassium hydroxide, shake vigorously, and allow to stand for several minutes. After closing the tube with a cotton stopper, heat 2 minutes in a boiling water bath, cool to 15° C., mix with 5 ml. of absolute acetone containing 1% *n*-propyl alcohol, and filter. Wash the precipitate with the acetone–*n*-propyl alcohol mixture and dry at 100° C.

ETHERS

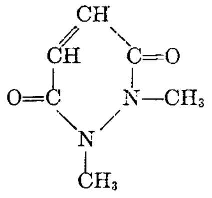
The source of error in the alkoxyl determination is the subject of several papers.

Simple sugars and related compounds such as cellulose derivatives yield volatile iodine-containing compounds (*53*), when subjected to the conventional methoxyl determination. The error is eliminated by using the trimethylamine absorber technique.

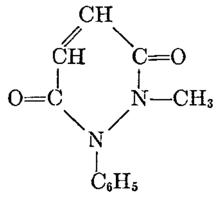
The use of sodium thiosulfate solutions to remove hydrogen iodide from alkyl iodides leads to erratic results (*41, 59*) because of interaction between the alkyl iodide and thiosulfate. Substitute washing solutions are suggested.

Methoxyl and ethoxyl groups may be determined simultaneously by conversion to the iodides, followed by absorption in isopropanolic trimethylamine (*54*). The tetramethylammonium iodide precipitates quantitatively; ethyl iodide is recovered from the filtrate.

Compounds such as 1,2-dihydro-1,2-dimethyl-3,6-pyridazinedione (II) and 1,2-dihydro-1-phenyl-2-methyl-3,6-pyridazinedione (III) undergo rearrangement to the *O*-methyl derivatives under the conditions of the Zeisel determination (*57*). Misleading and unreliable results may be expected when the Zeisel determination is applied to compounds of this type.



II



III

The use of fuming hydrogen iodide permits the determination of the ethoxyl group in alkyl ethoxysilanes (*20*).

The analysis of α-haloalkyl ethers (*130*) and α,β-dichloroalkyl, and α,β,β-trichloroalkyl ethers (*131*) is based upon their hydrolysis to a hydrohalic acid, an aldehyde, and an alcohol. The acid may be titrated with base or the halogen determined argentometrically.

OXIRANES

Dilute aqueous solutions of epoxides or glycol mixtures can be analyzed with an accuracy within ± 0.5% (*149*). The sodium hydroxide, liberated in the reaction of epoxide ring with sodium sulfite, is titrated with standard hydrochloric acid.

Glycidic esters react quantitatively with hydrogen iodide to produce iodine. Titration of the iodine with sodium thiosulfate provides a convenient method for the determination of these compounds (*35*).

PEROXIDES

Procedures for the determination of organic peroxide have been critically examined.

A comparison of the Wheeler iodide and stannous chloride chemical methods with the polarographic method (*115*) indicates that they yield identical results with pure peroxides. The polarographic method is recommended for impure samples because of its greater selectivity.

The arsenious oxide method of Siggia (*133*) was found, on the average, to give higher values (*112*) for peroxide than the Kokatnur-Jelling procedure (*78*). Modifications of the latter procedure when applied to the determination of benzoyl peroxide are presented.

PHENOLS

Ceric sulfate has been employed in the determination of phenol and phenol derivatives in both volumetric and gravimetric procedures.

Phenol is oxidized rapidly at room temperature by ceric sulfate (*140, 152*), whereas hydroquinone, pyrocatechol, resorcinol (*150*), phloroglucinol, and pyrogallol (*152*) require elevated temperatures for complete reaction. The reaction with cresol is incomplete. The excess ceric sulfate is determined by back-titration with ferrous sulfate.

An empirical gravimetric method (*140*) involves the oxidation of phenol to a solid product by the ceric sulfate solution, followed by filtration, drying, and weighing.

p-Nitro-*p*′-aminoazobenzene (*155*) has been recommended as an indicator for the nonaqueous titration of phenol in an ethylenediamine solvent.

The application of the Koppeschaar bromination procedure to the analysis of various nitrophenols (*66*) and 2,4-dinitrophenol–picric acid mixtures leads to erroneous results because of the replacement of nitro groups by bromine under the conditions of the procedure.

Other phenols and phenol derivatives (*1*) which have been determined successfully by this method are *o*-cresol, 4-chloro-2-methylphenol, and 2-methylphenoxyacetic acid.

In a comparative study of the iodine method and the coupling method for the determination of 2-naphthol in phenyl-2-naphthylamine and *N,N*′-di-2-naphthylphenylenediamine (*77*), the iodine method was found to be unsatisfactory, but the use of the *p*-nitrobenzenediazonium chloride procedure was successful.

Picrate ion may be determined gravimetrically (*104*) by precipitation with 4-octyloxyphenylguanidinium chloride followed by drying at 110°.

SULFENYL HALIDES

Kharasch and Wald (*73*) report the adaptation of an iodometric method for the analysis of 2,4-dinitrobenzenesulfenyl halides. Iodine, which is formed according to the equation

$$2\ ArSCl + 2I^- \rightarrow ArSSAr + I_2 + 2Cl^-$$

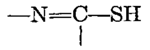
is consumed by the addition of excess sodium thiosulfate, the excess being determined by back-titration with a standard iodine solution. An accuracy within ± 1% is obtained if anhydrous media are employed.

SULFIDES

When the bromometric method of Siggia and Edsberg (*134*) is extended to derivatives of mercaptoacetic acid (*46*), the results are usually high, owing to sulfone formation, or reaction with the methylene group, whereas with 2-thiophenecarboxylic acid and derivatives, the reaction is incomplete. The stoichiometric value of bromine should be determined for each compound.

THIOLS

Mercapto derivatives containing the structure



$$—N{=}\underset{|}{C}—SH$$

are oxidized quantitatively by selenious acid (*40*). After reaction of the selenious acid with nitrogen heterocyclic compound or with the thiosemicarbazone, the excess oxidizing agent is determined iodometrically.

Thioacetals and thiolic esters can be titrated quantitatively at 30° to 40° in acid media with 0.1*N* bromide-bromate solution (*45, 47*) to a permanent yellow end point.

UNSATURATION

The usual procedure for the determination of olefins involves the addition of halogen or some other reagent to the double bond.

Rosin acids containing two double bonds react instantaneously with methanolic bromine solution (*64*). Dehydroabietic acid and tetrahydroabietic acid do not react under these conditions and dihydroabietic acid reacts only slowly.

Unsaturated amines (*138*) give unreliable iodine numbers because of the replacement of amino hydrogen by halogen. Satisfactory values can be obtained if the amine is first converted to its hydrochloride in an alcoholic chloroform solvent.

Arve (*5*) reports that mercurous acetate accelerated the addition of iodine to the double bond in determinations of iodine number.

Ethylenes and substituted ethylenes may be determined with an accuracy within 1 to 3% (*31*) by reaction with excess mercuric acetate. The excess is estimated by titration with propylene glycolic hydrochloric acid to a thymol blue end point. This method is superior to the Marquardt-Luce (*89*) alkimetric procedure when applied to the determination of vinyl acetate and allyl acetate.

Modifications are reported in the usual procedure for the determination of acetylenic compounds by precipitation with silver nitrate followed by titration of the hydrogen ion produced.

The use of saturated solutions of silver nitrate or silver perchlorate, to form a soluble complex with acetylene (*10*), permits analysis of acetylenic alcohols, hydrocarbons, carboxylic acids, and amines with an accuracy within 0.5%. The principal advantage is the elimination of the precipitate which interferes with the detection of the end point.

Another modification (*90*) employs silver benzoate in place of silver nitrate to form the insoluble silver acetylide, followed by titration of the benzoic acid produced.

Copper acetylide in the presence of other copper compounds is determined by decomposing the acetylide with potassium cyanide (*108*). The acetylene produced is determined in the usual way.

MISCELLANEOUS METHODS

MIXTURES

Mixtures of methanethiol, dimethyl disulfide, and dimethyl sulfide are analyzed by first absorbing the thiol in mercuric cyanide and the sulfide and disulfide in benzene. The methanethiol, upon absorption in 4% mercuric cyanide, is converted to mercuric dithiomethoxide [$Hg(SCH_3)_2$]. This latter compound can be determined either by weighing, iodometrically, or by analyzing the mercuric cyanide solution for liberated cyanide ion. The dimethyl disulfide in benzene is reduced to methanethiol and determined by a Volhard titration. Dimethyl sulfide is oxidized with bromine water and the liberated hydrogen bromide is titrated. Results are within 5% of the theoretical values. The method can probably be applied to homologous compounds (*129*).

Thiols have been determined in the presence of hydrogen sulfide by taking advantage of the difference in solubility of CdS and $Cd(SR)_2$ under conditions of controlled pH. From the iodometric determination of sulfide after separation of the more soluble $Cd(SR)_2$ and from the iodometric determination of total thiol (H_2S and RSH) on a separate portion, both sulfide and disulfide can be calculated (*30*). Disulfide in the mixture can be determined by modification of the zinc–acetic acid reduction method of Bell and Agruss (*15*).

A rather unusual method for determining acetic acid in acetic anhydride depends upon the reaction of triethylamine (in benzene) with the acid in the anhydride. The reaction appears to be as follows:

$$3HOAc + (C_2H_5)_3N \rightarrow (C_2H_5)_3N.3HOAc$$

A methyl red indicator is used, and the end point is taken as that point at which the titration sample has the same color as methyl red in benzene (*87*). The method is probably applicable to the determination of other acids in their anhydrides.

A procedure for sodium laurate in sodium lauroyl sarcosinate is based on the insolubility of lauric acid in diammonium phosphate. The lauric acid is then titrated with a cationic detergent using a difluorescein indicator (*63*).

The bromate-bromide method for phenols has been applied to certain alkylated phenol mixtures by taking advantage of the different number of ortho and para positions available for bromination in alkylated and nonalkylated phenols (*141*).

The effect of pH on the periodate oxidation of glucose and sucrose permits the analysis of mixtures of glucose, sucrose, and soluble starch. In the presence of a bicarbonate-buffered solution at 25° only glucose is oxidized by excess periodate. After removal of starch with cadmium hydroxide, the total of glucose and sucrose can be determined by periodate oxidation in acid solution. After the oxidations, the excess periodate is determined by titration with thiosulfate (*113*).

An evaluation of several methods for the determination of total peroxide, total aldehyde, and formaldehyde in solutions containing hydrogen peroxide, acetaldehyde, and formaldehyde (*122*) indicates that total peroxide is best determined by thiosulfate titration of the iodine liberated from glacial acetic acid–hydrogen iodide solution. Total aldehyde is determined by oxidation with hydrogen peroxide in the presence of a known amount of alkali. The acid formed from the aldehyde neutralizes part of the alkali. Titration of the excess base gives an indirect determination of the aldehyde. The study shows that formaldehyde is best determined colorimetrically with Schiff's reagent after removal of peroxides.

A rapid method for determining the carbon dioxide content of ethanolamine solutions consists of adding excess sodium hydroxide to the solution and back-titrating with 0.5*N* acetic acid (*162*).

SUGARS AND RELATED SUBSTANCES

Aldonic acids have been determined by titration with standard calcium chloride solution at pH 12.4. The method is based on the sequestering action of sodium gluconate (and related substances) for calcium in strongly alkaline solution. No indicator is used in the titration. The end point is a distinct and persistent turbidity. The methods appear to be applicable to quantitative differentiation between certain acids (*69*).

The calcium content of calcium pectate has been determined by titration with (ethylenedinitrilo)tetraacetic acid (EDTA). Pectate ions do not interfere. Calcium contents of precipitates containing 6 to 24 mg. of calcium (as calcium carbonate) were found to be proportional to the quantities of citrus pectin from which the precipitates came (*61*).

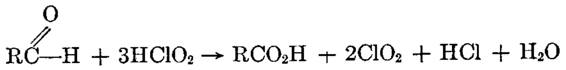
The method of Kline and Acree (*76*) (in which the aldehyde group is oxidized by hypoiodite) is reported to be subject to serious error when applied to mannose. The following modification has been suggested to overcome this difficulty (*29*).

To 0.225 gram of the hexose is added equal volumes of 1*N* sodium carbonate and 0.1*N* iodine (containing 60 grams of potassium iodide per liter). The mixture is kept at 20° for 30 minutes after the first addition of iodine solution and is then acidified with 6*N* hydrochloric acid and the excess is titrated with 0.1*N* thiosulfate solution, using a starch indicator.

The determination of mannose and mannans as mannose phenylhydrazone (*116*) has been modified so that the method requires less time (*117*).

In studies on the hydrolysis of dextran and the destruction of glucose (*34*) it has been found that essentially the same maximum yield of reducing sugars and concurrent loss of glucose are obtained whether the hydrolysis is carried out with 2 or 4*N* sulfuric acid at 100° or 1*N* sulfuric acid at 120°. The time necessary to reach the maximum yield varied, however, from 50 to 180 minutes. In comparing sulfuric acid and hydrochloric acid it was noted that the acids were equivalent on a molarity basis rather than on a normality basis with respect to time of hydrolysis and rate of destruction of glucose. A standardized procedure for analytical hydrolysis of different types of dextrans is given. It uses a 0.5% solution of dextran in 4*N* sulfuric acid heated at 100° for 75 minutes. The reducing power of the hydrolyzate is corrected for an approximately 4% loss of glucose during the hydrolysis.

The following reaction



$$RC(=O)—H + 3HClO_2 \rightarrow RCO_2H + 2ClO_2 + HCl + H_2O$$

takes place in phosphate buffer solutions at pH 2.4 to 3.4 at 50° By using a known excess of chlorous acid and determining the unused chlorous acid iodometrically, the reaction becomes the basis for a quantitative determination of glucose. The chlorous acid reagent decomposes during the reaction and an estimated correction must be made for this. The favorable stoichiometry makes the method useful for determining trace amounts of glucose. The method can presumably be applied to other aldoses (*81*).

Another method for determining reducing sugars (*110*) is based on the complexometric titration of cuprous oxide in alkaline solution. A solution containing complexon III [sodium salt of (ethylenedinitrilo)tetraacetic acid] as the complex-forming reagent is suggested.

For the determination of small changes in sugar concentration, two new procedures make use of the oxidizing properties of ceric perchlorate (*39*). In one of the methods the excess ceric perchlorate is decomposed with sodium oxalate, while the second method uses arsenite solution in the presence of osmic acid.

The availability and properties of sodium borohydride will probably lead to its quantitative application in a number of instances. One way in which this reagent has been applied is a gasometric method for reducing sugars (and other carbonyl compounds) (*137*). In this method an aqueous solution of sodium borohydride is standardized by measuring the volume of hydrogen evolved upon acidification of the solution. The sugar sample is treated with the borohydride solution and then, after the reduction is completed, the excess borohydride is determined by acidification and measurement of the hydrogen evolved. Reduction is complete in 20 to 30 minutes at room temperature or in less than 1 minute at the boiling point. An accuracy within 1% of the correct value has been obtained in the determination of glucose, fructose, galactose, lactose, and glucosamine. The method appears to be similar to that of Lindberg and Misiorny (*84*).

A method for fructose (*111*) depends upon oxidation with a known volume of alkaline copper solution. After the oxidation the solution is neutralized, treated with potassium iodide solution and starch indicator, and then titrated with standard thiosulfate solution to determine the excess cupric ion in the solution.

For the determination of starch in concentrations up to 250 p.p.m. a rather empirical dichromate oxidation method appears to be convenient. The oxidation is preferably conducted in an autoclave at 120° for 30 to 60 minutes (*128*). The unconsumed dichromate is determined either by addition of excess Mohr's salt and titration of the excess ferrous ion with permanganate or colorimetrically. The starch is reportedly oxidized to carbon dioxide, water, and stable compounds of low molecular weight. The method has also been applied to laurylamine acetate and to sodium oleate. It might be useful for routine determination of various other organic substances.

In the periodate method for determination of end-group values of polysaccharides it is necessary to use carefully standardized conditions (*96*). A modified procedure has been described in which the oxidation period is 30 hours. The formic acid determined in the titration is corrected for an average yield of 81% as determined with sucrose.

Ascorbic acid may be titrated directly with a solution of *N*-bromosuccinimide in the presence of starch and iodide as indicator. One advantage of the method depends upon the fact that *N*-bromosuccinimide selectively oxidizes ascorbic acid before other reducing substances. The other commonly used method for ascorbic acid uses 2,6-dichlorophenolindophenol and is limited by the presence of other reducing substances (*9*).

WATER

Improvement in the stability of Karl Fischer reagent is obtained by using methyl Cellosolve instead of methanol in preparing the reagent. This modification of the reagent also extends the applicability of the method by permitting a wider choice of sample solvent. In the determination of water in aldehydes and ketones, a solvent consisting of ethylene glycol and pyridine is advantageous. It eliminates the possibility of the reaction of methanol with carbonyl group to give an acetal and water (*107*).

A new reagent for the determination of water consists of a solution of sulfur dioxide and bromine in chloroform. It is used much like the ordinary Karl Fischer reagent, but is reported to be more stable and give results which are more nearly stoichiometric. The disappearance of the bromine color serves as a satisfactory end point. The main disadvantage of the new reagent appears to be that alcohols interfere in the water determination (*14*).

UNCLASSIFIED

Most of the methods which have been used for the determination of quaternary ammonium compounds are based on Auerbach's reaction (*6*), whereby certain acidic dyes react with quaternary ammonium compounds to give a product that is insoluble in water but soluble in certain organic solvents. Recent studies (*8*) of the shift in the absorption spectrum of bromophenol blue when cetyltrimethylammonium bromide is added indicate that the reaction probably involves complex formation.

Ascorbic acid reduces indophenols slowly, but air oxidizes them again. The following technique permits the titration of indophenol samples with a standard solution of ascorbic acid.

> Most of the ascorbic acid is added rapidly to the sample, and the solution is allowed to stand for 10 to 15 minutes with periodic shaking. The titration is then finished in 1 to 2 minutes with energetic shaking of the sample. (A preliminary titration is necessary to establish how much ascorbic acid to add in the first addition.) An inert gas atmosphere is not required. Ascorbic acid solutions are prepared by dissolving a weighed amount in 2% sulfuric acid prepared from boiled water. The solution is standardized with potassium iodate, or standardization may be omitted if sufficiently pure ascorbic acid is used. The solution keeps for only 1 to 2 days (*145*).

The reaction of 1-naphthylthiourea with bromine to give 2,4-dibromo-1-naphthylurea is the basis for a new determination of the former compound (*167*). The sample is dissolved in 100 ml. of acetic acid and 5 ml. of 25% hydrochloric acid and then titrated with $0.1N$ potassium hypobromite. In brominating the ring and reacting with the thiocarbonyl group a total of 12 equivalents of bromine are used per mole of sample. 2-Naphthylthiourea cannot be determined by this method because the analogous reaction in this case is too slow.

A new method for the estimation of xanthates appears to be dependent upon the favorable solubility of lead xanthates in benzene. The sample is treated with lead nitrate solution. The lead xanthate is taken up in benzene and washed with water to remove excess lead salts. The benzene layer is evaporated to dryness and the residue of lead xanthate is weighed. The weight of the residue is multiplied by the empirical factor 0.712 to find the weight of potassium xanthate in the sample (*88*).

The potassium xanthates of 12 common alcohols can be titrated potentiometrically or in the presence of an indicator in glacial acetic acid, even though the xanthates are unstable in this solvent (*17*).

The explanation for this apparent anomaly lies in the fact that the $ROCSS^-$ ion either neutralizes a solvated proton or acquires the hydrogen from a neutral acetic acid molecule. The ROCSSH thus produced decomposes, and the acetate ion neutralizes a free solvated proton, thus keeping the reaction stoichiometric.

Ion exchange resins continue to find many applications in analytical chemistry. The following procedure is used in determining both sulfur and barium in barium sulfonates (*24*). The barium salt, dissolved in water, is passed through a small cation exchange column. The free sulfonic acid is eluted and oxidized to sulfate by one of the customary methods. The column is then eluted from the other end with 12% hydrochloric acid, and the barium in the eluate is precipitated with sulfuric acid.

The number of $—CH_2CH_2O—$ groups in ethylene oxide condensation products can be determined by treatment of the condensation product with a known excess of potassium ferrocyanide, the precipitate which forms is removed by filtration and the excess ferrocyanide in the filtrate is titrated with zinc sulfate (*124*). Approximately 0.7, 1.0, and 1.5 moles of potassium ferrocyanide react with the condensation products of nonylphenol with 6, 9, and 12 moles of ethylene oxide.

Tetramethylphosphonium chloride can be determined in aqueous solution by the addition of an excess of chloroplatinic acid. After evaporation to dryness, the residue is washed with alcohol, filtered, washed, dried, and weighed. The accuracy is within 0.5%. Moderate amounts of calcium, magnesium, and strontium ions do not interfere, but ammonium, potassium, rubidium, and cesium ions are also precipitated. Alternatively, the tetramethylphosphonium cation can be removed with a cation exchange resin, and the hydrochloric acid can be titrated (*3*).

Hexahydro-1,3,5-trinitro-*s*-triazine (RDX) is apparently titratable as a tribasic acid if the sample is dissolved in dimethylformamide and titrated with sodium methoxide in a benzene-methanol solvent (*71*).

Titanous chloride has been used for the reduction of the nitro group of nitroguanidine and conditions have been worked out to make this method essentially quantitative (*23, 146*).

Other investigators (*143*) have determined the nitrogen in nitroguanidine and in nitrocellulose by using the sample as the nitrating agent for the transnitration of salicylic acid. The 5-nitrosalicylic acid thus formed is estimated by reduction with an excess of standard titanous chloride.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Biochemical Analysis

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BEFORE proceeding to the review of major developments in biochemical analysis for the past 2 years, it is well to state the general requirements and restrictions of biochemical analysis, in contrast to those of analytical chemistry. "Biochemical analysis is inherently a more complex and broad field than classical chemical analysis. Because the major activity of the biochemist is always to find out what and how much, biochemical analysis actually must include virtually every biochemical technique" (*73*).

Because the biochemist directs his analysis toward the understanding of structure and content of biological units, his analyses must be accomplished on samples which vary enormously in interfering substances, such as proteins, nucleic acids, and lipide. Rarely can an analysis be transposed without modification from analytical chemistry into biochemistry; this is apparent even in the case of the relatively simple technique of flame photometry.

The biochemist manifests proper concern with fractionation and sample preparation before analyses are attempted, as the analyses have meaning only in their biological context. Thus, developments in fractionation procedures are eagerly accepted, whether the fractionation is that of simple molecules, macromolecules, or cellular particulates.

The recent development of methods for disassembly and reassembly of the molecular units of collagen (*52*), and a similar reassembly of isolated nucleic acid and protein subunits to form infectious tobacco mosaic virus (*46*), are of extreme importance. The breakdown of other macromolecules to molecular subunits would ease the restrictions on present attempts to relate analytical units (amino acid, nucleotide, etc.) to polymer structure. One consequence would be to expect the amino acid analysis of the virus protein to fit a subunit array of 10^4 weight, rather than the unmanageable total protein weight of more than a million. Another consequence is that the hydrophobic residues and charged groups of such proteins cannot be arranged in random fashion; the concentration of charges, especially, must be stereospecific to allow polymerization to occur. Thus, the directed crystallization of protein molecules, such as collagen, must result from highly "ordered" shapes and surfaces of the fundamental units. A development represented by the findings described above is that the electron microscope has matured as an analytical instrument. It now appears the instrument of choice for examination of biological macromolecules, prior to the use of the analytical ultracentrifuge, or light-scattering.

The review period presents a picture of maturation of ion exchange, paper chromatography, and zone electrophoresis. New methods of separation or analysis have been less apparent, greater emphasis being placed upon the correct use of consecutive fractionation methods followed by simple analysis. A great tendency exists at present for workers to consider all chromatography as incidental to the biochemical study concerned. Any scheme of fractionation is the servant of the process of discovery of biochemical concepts. However, the way of a reviewer is eased if brief attention is paid to novel fractionations or techniques, as incidental findings.

Instrumental analysis continues to emphasize absorptiometry. Titrimetry is neglected, with little usage of potentiometry, amperometry, or high frequency end points. Coulometry or polarography is rarely used. Respirometry, with the techniques of Warburg or Holter and Linderstrøm-Lang, is used only when less sophisticated procedures have failed to yield the desired data.

PREPARATIVE METHODS

CHROMATOGRAPHY AND ION EXCHANGE

Chromatography continues to bring ease and convenience to fractionations in every field of biochemical research. Adsorption chromatography, partition, and ion exchange have achieved various degrees of success. Universal use of paper partition chromatography occurs throughout the biological sciences. Ion exchange is perhaps second in importance, as it allows a rational separation of contaminants, or the fractionation of similarly charged isomers or related compounds. The burden of review of these fields is eliminated by the recent appearance of a variety of monographs (*8, 18, 26, 29, 85, 97, 145*) which correlate past work and project the future advances of chromatographic methods.

The prior developments in the field of ion exchange are reviewed by Lederer and Lederer (*85*), in the previous review (*74*), and in the practical manual by Brimley and Barrett (*18*). In the experience of this reviewer, the number of papers devoted to separations or new uses of resins has fallen to a small fraction of the papers appearing during 1952–54.

Absorbents and Resins. The utility of chromatographic separations is completely dependent upon the existence of material which allows the desired separation, whether the separation uses reversible adsorption, partition between stationary and mobile phases, or ion exchange.

Paper chromatography and ion exchange suffice for the major separations of amino acids, carbohydrates, or nucleotides. The use of natural adsorbents is minor at the present time. Silica gel or alumina is used as column adsorbent in a few specialized separations of steroids (*20, 55, 82*) or carboxylic acids (*19, 121*). The volatile organic acids will be separated in the future by gas phase chromatography, rather than by partition on silica. A new adsorbent for mixtures of polystyrene or poly(vinyl acetate) is urea (*69*), with benzene as the developing solvent. Though the process is of no immediate use to biochemists, a certain interest in the work remains. The question arises: Is it possible to form a hydrocarbon-specific urea complex, capable of repeated use in column separations, along the lines described by Marschner (*94*)?

The resins in major use in ion exchange continue to be the commercially available styrene-base resins, either sulfonated—e.g., Dowex 50 × 8—or containing a particular quaternary amine group (Dowex 2 × 8). The designation ×8 identifies the percentage of divinylbenzene in the original polystyrene latex. This degree of cross linking allows approach of small ions, without noticeable quantitative adsorption of proteins or other macroions. A popular resin for protein separations continues to be the self-buffering carboxylic resin, IRC 50, of unspecified resin matrix. Attempts to produce resins active in oxidation-reduction continue, the latest product being a polythiol resin, prepared from polystyrene (*50*). Resins of great interest to biochemists are formed by treating cellulose so as to introduce a number of phosphoryl groups for cation exchange, or esterified taurine (free monoalkylamine groups) for anion exchange (*70*). Phosphorylated cellulose in the form of an endless cotton belt has been proposed as a continuous cation exchanger (*103*).

Finely powdered cellulose has been modified by Sober and Peterson (*126*) to provide either anionic carboxyl or cationic amine groups. These workers are proceeding toward "resins" specifically tailored to allow the ion exchange of proteins or other macro ions. The preliminary results obtained by several groups of workers are described in a following section.

Amino acids may now be fractionated by gradient elution from the new Dowex 50 × 4 resin (*101*), or on Zeokarb 225 (*21*). Both resins have sulfonate groups and a polystyrene base, though the latter has unspecified cross linkage. Hirs, Moore, and Stein (*56*) describe a preparative fractionation of amino acids which uses both Dowex 1 × 8 and Dowex 50 × 4, with acetic and hydrochloric acids as eluents. The volatile acids simplify the later steps toward preparation of pure amino acids. A disturbing finding, not yet confirmed, has been the discovery of partial separation of carbon-14 amino acids from their natural counterparts, during fractionation on a Dowex 50 column (*107*).

Solute Recovery Techniques. The common method of solute recovery, in methods based on adsorption or partition, is the use of solvent mixtures of an indifferent solvent and a specific solvent. A useful procedure is that of continuous variation of solvent composition, gradient elution. Gradient elution using solvent pairs is a common procedure (*2, 19, 47, 74, 82, 121*). The technique may also be used to supply graded increases in acid (*19*) or buffer electrolyte concentration (*101*) in ion exchange. Drake (*36, 38*) indicated, from theory and experiment, that gradient elution need not increase resolution between peaks of material, although the peaks are sharpened and more symmetrical.

Column Monitoring. Anyone attempting column chromatography of amino acid or nucleotide mixtures is soon aware of the necessity for rapid proximate analysis of the multitude of fraction volumes obtained, before serious analysis is possible. If the peaks may be identified by any method, the number of serious analyses may be cut by 2- to 100-fold. Ideally, one method should suffice for the location of all volumes representing a "peak," following which these volumes could be combined, adjusted to known volume, and analyzed for the actual average solute concentration. In preparative work, it is sufficient to judge the identity and limits of a particular peak, thus collecting the pure solute for further purification or crystallization.

Light absorption, in the visible or ultraviolet, remains one of the most sensitive and convenient methods of monitoring column output. One of the most ambitious attempts to separate nucleotides routinely, using adsorbance ratios for 260/280 mμ, is that of Deutsch, Zuckerman, and Dunn (*33*). These workers relied on electronic decision for delivery of the nucleotide fraction to the correct receiver. For the analysis of ribose nucleic acid, for example, the curve of absorbance *vs.* fraction number obtained from the monitor would represent the quantities of nucleotide present, after integration under the peaks (*26*). Some correction for background absorbance may be necessary before integration. These analyses are unique in their simplicity once precise fractionation is obtained. As nucleoside mono- and polyphosphates are important compounds in carbohydrate metabolism and nucleic acid synthesis, such fractionations continue to provide information of dynamic importance (*59, 118*). Where absorbance measurements are impossible or inconvenient, monitoring is sometimes possible on the basis of increments of refractive index (*68, 138*), conductance (*34, 37*), pH (*64, 66, 147*), or dielectric constant (*83*).

Unpublished work in this laboratory, as well as recent reports (*25, 134*), indicates that the fraction collection and analysis may be simplified, on a small "pilot" column basis, at least. The innovations consist of collection of one or several drops of eluted fraction in a single area of filter paper tape. The tape may be continuously moving (*25*), or a number of drops may be collected and evaporated in one region, the paper moving discontinuously (*40*). Following collection, the amino acid spots may be detected with ninhydrin treatment and color development in a hot paraffin bath (*25*). If nucleotides are fractionated, the regions of interest may be detected by ultraviolet reflex photography (*40*). Detection of radioactive peaks on such paper tapes is possible, using techniques already developed for paper chromatography (*42, 53, 88, 144*).

CHROMATOGRAPHIC SEPARATION OF PROTEINS AND NUCLEIC ACIDS

In keeping with the present trend to focus once more upon the solutes being fractionated, rather than on detail of fractionation, data bearing on the purification of several natural macromolecules are grouped together. It was reported in 1954 (*74*) that only ribonuclease, lysozyme, and one cytochrome had yielded to fractionation. Ribonuclease was capable of isolation and fractionation by either partition or ion exchange. The other proteins were adsorbed and eluted from the carboxylic resin, IRC 50. Some purification of thrombin occurs during similar treatment (*111*). Separation of two bovine hemoglobin derivatives, the carbonyl derivative and the oxidized methemoglobin, is also possible (*10*). The rational separation of histones on the barium ion form of the same resin has been accomplished by Crampton, Moore, and Stein (*30*). These authors find three types of histone from calf thymus which can be identified on the basis of arginine or lysine content. Several similar histones exist in calf liver, or in guinea pig testes. In one of the first reports on the use of the resin, treatment with Dowex 2 has resulted in purification of several phosphatases (*11*).

Partition on Celite has separated various immune globulins from bulk γ-globulin (*108*). Partition on silane-treated Celite has yielded purified insulin A and B polypeptides (*3*). Fractionation of a variety of dissimilar proteins has been attempted by Tiselius (*136, 137*). He used a simple calcium phosphate gel, and proteins ranging from egg albumin to tobacco mosaic virus. The results are preliminary, for simple known mixtures, but they promise a separation method for proteins. Tiselius regards the method as a "gentle" one, as no organic phase is present in the adsorbent. It may be possible to form the gel about molecules of a desired protein, so as to mold the gel for later specific use. The synthesis of several cellulose derivatives as adsorbents for protein purification has been reported by Sober and Peterson (*126*). These workers find that their low-capacity adsorbents are equipped to provide separation of numerous enzymes from heart, spleen, or kidney extracts. They also report (*106*) that the various serum proteins are separated. Peterson, Wyckoff, and Sober (*106*) recognize an element of ion exchange in these fractionations, although adsorption by hydrogen bonding must also operate. These new "resins" have been used also in nucleic acid separations, as described below.

Many workers in nucleic acid chemistry consider that the ribose (RNA) and deoxyribose (DNA) nucleic acids are classes rather than individuals. Until the present time, nothing approaching a separation of individual ribose or deoxyribose nucleic acids has been reported. The preliminary reports (*6, 14*) may represent a beginning to such fractionations. The first workers (*14*) used a cellulose amine resin formed from cellulose, epichlorohydrin, and triethanolamine, synthesized by Sober and Peterson (*126*). The amino groups are contributing a certain amount of ion exchange; capacity may be as low as 0.2 meq. per gram. Various ribose nucleic acid specimens provided a different spectrum of eluted fractions, with recoveries of 90 to 100% of the ribose nucleic acid taken (*14*).

Comparative fractionation attempts for deoxyribose nucleic acid on Dowex 1, Amberlite 4B, and two modified celluloses have shown the superiority of the same cellulose amine resin (*6*). Thus far, the only test applied is the reversibility of adsorption of the long fibrous deoxyribose nucleic acid molecules to the resin. The first true fractionation of one deoxyribose nucleic acid molecule from another may be realized during the purification of a "transforming principle," a deoxyribose nucleic acids of pneumococcal origin which exerts a genelike action on other pneumococci. Here, success in fractionation of deoxyribose nucleic acid will be demonstrated, if increased biological activity is achieved through fractionation of analytically similar deoxyribose nucleic acids. Some purification of one "transforming principle" has been achieved using a column of Celite-albumin mixture as the adsorbent (*87*).

PAPER CHROMATOGRAPHY

The review of this field 2 years ago required consideration of hundreds of articles, just to outline the invasion of all fields of biochemistry by the technique. One may report at this time that the diverse growth of the method is complete; few chemical compounds refuse to separate by the technique, with the exception of the proteins and nucleic acids. The apparatus used may vary from a covered glass jar to a shiny Chromatocab. The all-glass or polyethylene atomizers for the inevitable spraying of corrosive test reagents are now cheap and commonplace. Compounds may be chromatographed on one of several filter papers, with one of many solvent mixtures, as the native compound, or as a derivative. The experience in this field is now amply described by monographs or manuals (*8, 29, 85, 89, 97, 145*). A few recent articles on paper densitometry or detection of zones are reviewed.

The natural desire to extend paper chromatography from isolation-identification to an absolute determination has not been realized. The complexity of absorbance measurement directly on filter paper is outlined by Price (*109*). Densitometers of several types are available, finding use also in the technique-related field, paper electrophoresis. Densitometry does suffice to establish approximate quantity and relative concentration of plasma proteins, after electrophoresis and staining (*8, 28, 42, 67*). The technique may also serve for the location and identification of ultraviolet-absorbing compounds, such as the barbiturates (*91*), or purines and adrenal steroids (*110*). McReady and McComb (*93*) suggest that reflectance, rather than absorbance, may be used for the estimation of carbohydrates after chromatography. They use a commercial densitometer and attain a precision of 5% in the determination of 50 γ of the common hexoses.

It is seldom possible to detect zones on a chromatogram without the destruction of the compounds involved. Intrinsic light absorption provides an exception to this rule. Two new methods based on capacitance or conductance are also capable of locating zones without destroying the compounds or ions responsible. Blake (*7*) suggests the application of radiowaves at 1-mc. frequency to the paper by two electrodes to outline the zone. The current passed by the electrodes is rectified and read on a suitable meter. The method has general application where electrolytes or polar compounds are concerned. An alternative method has been proposed for detection of electrolytes in inorganic separations. This method (*35*) suggests passage of direct current between rollers which come in contact with the chromatogram.

GAS PARTITION CHROMATOGRAPHY

This technique extends the advantages of chromatography to micro samples of compounds commonly separated by complex distillation procedures. The subject was reviewed briefly in 1954 (*74*), but the importance of the method is considered such that a few references are repeated to permit more complete coverage of the subject.

The method, developed by James and Martin (*62*) for analysis of volatile acids, requires a passage of the solute mixture at "distillation" temperature through a 4-foot column packed with Celite moistened with a high-boiling silicone or other solvent. The necessary mobility is provided by a nitrogen stream. The fractions are carried into a collection vessel, where they are periodically titrated with standard alkali to a photometric end point. A plot of microequivalents of alkali required against time of titration would provide a stepped or smooth sigmoid curve, whose midpoint location in time corresponds to the appearance of particular acids in the observation vessel. The method has also been applied to amines and pyridines (*60, 65*), and presumably could also be extended to substituted phenols. The aromatic amines may be separated on Celite-paraffin (*61, 63*).

With masterful understanding of the principles involved, James and Martin (*63*) have extended the procedure to include separation of the aliphatic hydrocarbons and the aromatic hydrocarbons. The separation of the hydrocarbons required the development of a new detection device, as the common thermocouple device did not prove sensitive enough for their purposes. The general detector is an automatic gas density balance (*63*). Martin considers the advantages of gas phase chromatography to be: negligible solute viscosity, rapid equilibration, and simple solute detection. Other workers have provided an additional detection device, the infrared analyzer, following oxidation of the fractions to carbon dioxide (*96*).

Undoubtedly, the general procedure described is capable of greater refinement and extension of application. Even in its present stage of development it is capable of separation of the isomeric hydrocarbons from pentane through octane, in an analysis requiring about 30 minutes. One does not wonder that commercial units embodying these separation principles are already announced by Burrell, Perkin-Elmer, and Podbialniak in this country. Chromatography has invaded one of the last untouched fields of chemistry, with a tool capable of providing rapid microanalyses of complex mixtures on a "simple" apparatus.

ZONE ELECTROPHORESIS

This field, like that of paper chromatography, has "come of age" with the appearance of reviews (*81, 105, 125*) and monographs (*8, 86, 92*). The necessary growth of knowledge concerning apparatus and technique has been more restricted, since the specifications for routine application of the method are more rigorous than those required for paper chromatography. The techniques used by Durrum (*8*), McDonald (*92*), and Kunkel (*81*) remain distinctive, just as the commercial assemblies are produced in all three forms. The three variants use the filter paper in a taut, inverted V form, a taut horizontal form, or a horizontal form between pressure plates of glass. An apparatus using the paper in the last arrangement with pressure between glass cover and metal lower support is described by Werkheiser and Winzler (*142*). The recently described apparatus of Crestfield and Allen (*31*) represents a very useful form of the horizontal, glass-supported apparatus. It routinely uses water cooling, which allows higher voltage gradients and shorter times of separation. At the end of a run, hot water is directed into the cooling manifold to dry the paper in situ.

All forms of zone electrophoresis differ from the "ancestral" free-boundary technique, in that the fractions are obtained free of the other compounds of the mixture, given sufficient time for separation. The advantages of the new approach include: inexpensive apparatus, isolated fractions, micro quantity or micro concentration scale, little time and effort per run. The disadvantages include: mobility values are not absolute, though standards can be run simultaneously; adsorption on the anticonvective material is possible; electro-osmosis must be measured under the actual pH conditions used.

The importance of electro-osmotic effects is emphasized by Strain (*129*). This author uses hydrogen peroxide or hydroquinone as indicators of movement. Caffeine has been proposed as a standard for measurement of electro-osmosis, with picrate ion providing a mobility standard over the pH range, 4 to 10 (*31*). These substances are located on ultraviolet "prints" of the dried paper.

The amino acids and peptides may be separated by zone electrophoresis into acidic, neutral, and basic fractions (*51, 143*); the three fractions are commonly subjected to paper chromatography thereafter. The unusual ninhydrin spray may be used for their detection. The serum proteins and hemoglobins are separable, according to their differing net charges (*8, 28, 67, 72, 81, 92, 115, 117*). The proteins are usually stained with a mixture of mercuric chloride and bromophenol blue. The adsorbed dye may be eluted from cut fractions, or the dye-protein spots may be scanned with visible light (*28, 115*).

Nucleotides, nucleosides, and free purine or pyrimidine possess charge in sign and degree conferred by nucleotide phosphate, or by the amino and enol groups of the bases. These materials may be separated by one of the types of apparatus described. The particular zones are located by ultraviolet "prints" (*32, 125, 141, 142*). The nucleotides of the ribose nucleic acid in single nerve cells have been determined by Edström and Hydén (*43*), using electrophoresis on a rayon fiber, followed by microspectrophotometric estimation of the isolated nucleotides.

Presumably, the separation of carbohydrates (as borates) and aldehydes or ketones (as bisulfites) is also possible by electrophoresis as by ion exchange. A stimulating article on the separation of amylase-digested amylose has appeared (*102*). These authors were able to obtain separation in agar gel of the various fractions of the degraded amylose, using the familiar iodine complexes of these polymers as the migrating species.

The combination of electrophoresis and descending chromatography has not received the wide use accorded the single techniques. Most devices available for this continuous separation are similar in principle to the original ones described by Durrum (*41*) and by Strain (*130*), with two commercial models in production. Here a hanging paper curtain is used. Modified apparatus of the same type has been described (*58, 116*). The technically difficult apparatus described by Svensson and Brattsten (*132*) continues in use for the fractionation of serum proteins in quantity (*13, 16, 17*). This apparatus employs a vertical tank filled with fine glass powder, with careful delivery rates of buffer into and out of the tank.

Gradient Electrophoresis. A new approach toward preparative electrophoresis has been taken in the past 2 years by Kolin (*76–79*) and Svensson (*131, 133*). Kolin has approached the problem of separation of proteins from a physical point of view. He already has achieved rapid separations in a type of free-boundary apparatus, through the use of density gradients to ensure the maintenance of any protein zone obtained. A pH gradient may be superimposed on the density gradient as well. The proteins taken (hemoglobin, catalase, cytochrome c) are separable on the basis of migration in a pH gradient until the isoelectric pH is reached (I spectra), or on the basis of differing mobility (M spectra).

Since Kolin is using a narrow zone of application and migrations of about 1 cm., the separations are extremely rapid. The difficulties which exist during present use of the apparatus are several: location of zones, careful removal of a zone unmixed with other zones, surety that the particular pH gradient is acceptable to the desired protein without denaturation. Undoubtedly, ways will be found to circumvent these difficulties. One could separate proteins from a complex mixture into acidic, neutral, and basic fractions. Thereafter, in a shallow pH gradient, the exciting method of migration of proteins to their individual isoelectric zones could be applied.

For electrophoresis in columns of starch or in a density gradient, several assemblies have been devised (*81, 131*). The apparatus developed by Svensson and Valmet (*133*) has been designed to deliver the isolated fractions by gravity flow to an automatic fraction collector, after the electrophoresis. Sorof, Ott, and Young (*127*) propose the use of the conventional Tiselius apparatus for density gradient electrophoresis. Such use is possible where the apparatus is not in continuous analytical use. The schlieren system should aid greatly in the location and removal of particular fractions.

DETERMINATIVE METHODS

Advances in methodology for the determination of individual components of biochemical systems have not kept pace with the advance in fractionation techniques. This trend is logical, as the precise fractionations, now commonplace, demand less of the determinative methods. The widespread use of a few "family reactions" is apparent now, as it was in 1954 (*74*); thus a single ninhydrin photometric procedure suffices to determine all α-amino acids, once fractionation has occurred.

ABSORPTIOMETRY

The determination of materials by quantitative absorbance measurement in the ultraviolet (*90, 120*) or visible (*57*) spectral regions continues to be of paramount importance. The trend is toward automatic determination of spectra by various recording spectrophotometers. More routine control of quantitative methods will result, as small spectral regions are identified as a means of determination of one component in complex mixtures.

As in 1954 (*74*), the use of infrared absorption continues to be an isolated special field. The use of infrared spectrophotometry has been discussed in a recent monograph on optical techniques (*24*). The findings regarding the detailed structure of biological macromolecules, such as deoxyribose nucleic acid (*9*) are of great interest. Infrared spectra will continue to provide information regarding "fine structure" of such polymers.

New Apparatus and Techniques. The announcement in 1954 and 1955 of three new recording spectrophotometers illustrates the modern trend toward automatic spectrophotometers of reasonable price and high routine accuracy. The near-infrared region, to about 3 microns, may be used for analysis in the Beckman instruments. The availability of the Keston (*71*) polarimeter attachment for the familiar DU spectrophotometer should increase the use of polarimetric analysis on a micro concentration scale, with small sample volume. More data regarding the optical rotation and rotatory dispersion of biochemical compounds should appear (*15*).

Cuvette modifications which serve to increase the utility of absorptiometry include the use of Teflon inserts for microvolume absorbance measurements (*123*), an anaerobic cuvette with provisions for additions or transfer of solutions (*84*), and half-height cuvettes for alternate pH adjustment and absorbance reading (*39*).

The extension of absorbance measurements to include turbid systems, such as cell suspensions, is possible in several ways. Shibata, Benson, and Calvin (*124*) use the expedient of interposing a diffusion plate, such as filter paper, following the emergent beam from the cuvette. Presumably the plate serves as a source of uniformly diffused light for the photomultiplier or other phototube. Bateman and Monk (*4*) approach the turbid systems from the standpoint of uniform reflectance of light not absorbed by the system. These authors use a round quartz flask, surrounded with magnesium oxide, as their sample holder. Light enters in one dimension and reaches the phototube after multiple reflections.

The quantitative measurement of fluorescence in biological systems has been used as a means of estimation of thiamine (*54*) since 1939. However, fluorescence has not been used as a general method of investigation until the present time. Two types of instruments have been developed which use the extreme sensitivity possible with the method (*12, 135*). The first instrument uses filtered light (360 mμ), detects the light emitted by the solute with a photomultiplier tube, amplifies the signal through a number of stages, and uses a variable sensitivity recorder. These authors estimate that the method is a thousandfold more sensitive than corresponding absorption methods. They report studies of the combination of flavin mononucleotide with the protein portion of Warburg's "old yellow enzyme." The free flavin is fluorescent; that combined with protein is not. The second instrument is a true spectrophotofluorometer (*12*), since the activation light is supplied by one monochromator and the emitted light is subjected to refraction in a spectrograph before intensity measurement. Udenfriend, Weissbach, and Clark (*139*) indicate that a large number of organic compounds, varying in complexity from aniline to morphine, are determinable in the instrument. Such determinations would be subject to interference by other fluorescent solutes in the analysis mixture. A second report indicates the determination of serotonin (5-hydroxytryptamine) in blood platelets by quantitative fluorescence. The estimation of less than 0.1 γ of the compound was possible.

The application of absorption, fluorescence, and other optical methods to the location and estimation of important compounds in tissue sections has been described by ten authors, under the editorship of Mellors (*99*). A second section of the book is devoted to methods using electrons and other radiation. The power of the fluorescence microscope is shown by the report of Mellors and others (*100*) on the location of antibody-antigen complexes at the site of pathological action. Localization in the glomeruli of rabbit kidney precedes the development of glomerulonephritis in these animals.

OTHER METHODS OF ANALYSIS

It is beyond the scope of this review to discuss the methods required for those determinations necessary to only a few analysts. Rather, the time and effort have been directed toward recognition of approaches or methods which promise general use in biochemistry.

Titrimetry. As indicated in the introduction, the use of methods based on titration (*44*) has been negligible in the usual organic analyses done by the average research biochemist. The exceptions to this would include any method which determines the appearance or disappearance of hydrogen ion as an indirect estimation of ammonia, or of the compound concerned. Similarly, the determinations of two biological cations (calcium and magnesium) frequently use titrations, with Versene as titrant (*112*). Alternative procedures, using the same reagents, may be absorptiometric (*1, 146*).

The Versene titrations or their absorptiometric equivalent will remain important in biochemistry. Their utility will be for the determination of micro quantity of bound metal ions which have been found as necessary components of important enzyme systems. Many of these ions can now be determined by the application of indicator titrations, using an azo-dye end point, and a chelating agent as titrant (*23, 95, 112, 122*).

Because many biochemists are no longer satisfied with subjective indicator end points, the trend is either to determine such ions as calcium by spectrophotometry of the calcium-dye chelate (*146*), or to determine the ion by Versene titration to a particular absorbance end point (*95, 122*). Such developments have led to the design of photometric titration units (*122*), or to the use of assemblies which permit titration in the confines of the cell compartment of the DU spectrophotometer (*75*) or of the Model B instrument (*45*). Photometric titrations are capable of general use, without excessive investment in capital equipment (*140*). Thus such titrations may be used for determination of a weak acid or for estimation of ionization constants (*49, 113*). The coulometric generation of oxidative titrant in such titrations is also possible (*45*).

Coulometric generation of titrant (*5, 119*) or the newer amperometric or voltammetric methods of end-point detection (*80*) have found few important uses in biochemical analysis. Such neglect of chemical advance is temporary, as acceptance awaits merely the need for the use of such methods, in preference to existing methods. Thus, polarography, which can discriminate among the related coenzymes (*22*) (diphosphopyridine nucleotide, triphosphopyridine nucleotide, and niacin mononucleotide), must still compete with existing methods, such as that based on chromatography followed by absorptiometry (*85*).

SOURCE MATERIALS FOR BIOCHEMICAL ANALYSIS

While time does not permit individual identification of chapters or authors, recent years have seen the appearance of a number of book serials devoted to the methodology of organic analysis (*98*), and biochemical analysis (*48*) for medical research (*128*) and for clinical chemistry (*114*). The three volumes on "Physical Techniques in Biological Research," devoted to optical techniques (*104*), physical chemical techniques, and cells and tissues, should prove of lasting value to the harassed research worker. The four-volume set of "Methods in Enzymology" (*27*) should prove of universal value, as the ideal is to present the method in enough detail to make repetition possible without return to the original literature. The published volume of this set provides a large section on preparative procedures for enzymes. This section includes authoritative summaries by various authors of advances in tissue homogenate preparation and differential centrifugation of cell particulates. For this reason, the subject of differential centrifugation is not reviewed here, although the older literature was covered in previous reviews of this series.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Distillation Analysis

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THE pace of advance in analytical distillation seems to have slowed, compared with earlier periods (*80*). The most exciting development, vapor-liquid chromatography, is not even recognized by some workers as distillation. Within the scope of conventional analytical distillation, the developments have been mainly in improving and extending previously described ideas and applications; the more important of these are indicated in the following sections. Particularly significant are the advances in multistage countercurrent molecular and low pressure distillation apparatus, and in micro distillation methods. There have also been some good papers on precision laboratory fractionation. Mair's use of fluorochemicals as azeotrope formers with hydrocarbons is also worthy of special mention (*60*).

VAPOR-LIQUID CHROMATOGRAPHY

Vapor-liquid chromatography involves establishment of composition differences between a liquid phase on the surface of a stationary solid adsorbent, and a moving vapor or carrier gas phase. The liquid phase of the mixture undergoing separation is partially converted to vapor of different composition from the liquid by the flow of carrier gas, as in steam distillation, desorption, and other examples of codistillation. A resemblance to extractive distillation arises from the presence of a purposely added nonvolatile liquid on the surface of the adsorbent, in which the components of the mixture being separated are dissolved. The presence of the purposely added nonvolatile liquid, and the adsorbed character of the liquid phase, undoubtedly result in major changes in relative volatilities, and are probably the chief basis for the remarkably sharp separations that are achieved. The vapor-liquid nature of the separation system allows the principles of distillation or desorption to be applied. In doing this, it must be recognized that vapor-liquid chromatography lacks the countercurrent flow characteristic of conventional distillation and absorption or desorption. The very small samples necessarily used make the process an example of microanalysis, and limit its use to cases where samples of the separated materials are not desired after analysis. A limited number of papers have been published on this new technique, and on the related process of gas chromatography (*4, 41, 43–46, 58, 65, 72*).

LOW PRESSURE DISTILLATION

Mair, Pignocco, and Rossini (*61*) have described in detail a relatively simple 50-stage apparatus for distillation at 0.01 to 0.1 mm. of mercury with countercurrent flow of the vapor and liquid streams. The important feature of this apparatus is that there is no pressure drop from one end of the apparatus to the other. The equipment consists essentially of a slanting tube with baffles located inside along the heated bottom side to prevent too rapid flow of liquid, and a cooled tube inside along the top, on which vapor condenses before it passes into collecting tubes that direct it to a point in the liquid stream, higher up in the tube. Vertical baffles in the vapor space prevent diffusional mixing in the lengthwise direction. Arrangements at the ends provide for introduction of reflux. The liquid throughput is only 80 ml. per hour, so at a reflux rate of 55 to 1, many hours of operation are needed to distill a charge of several liters. Typical results on the distillation of C_{17} to C_{25} paraffin mixtures are given. Aldershoff (*1*) has described a somewhat similar apparatus for use at lower pressures, in the molecular distillation range.

Melpolder, Washall, and Alexander (*63*) described a different multistage, countercurrent distillation apparatus for the molecular distillation pressure range. This apparatus consists of 20 glass molecular stills of conventional design, interconnected in series in such a way as to provide the desired countercurrent operation. With 19 small and one large (1250-ml.) stage, the total capacity is 1500 ml. Tests with 2-ethylhexyl phthalate–2-ethylhexyl sebacate showed 0.8 theoretical plate per stage. Automatic safety controls permit operation unattended for long periods. A single distillation is stated to require of the order of 24 hours. Earlier patents issued to Burrows (*13*) describe a somewhat similar apparatus.

Cantoni, Feldman, and Orchin (*17*) have described the use of repeated molecular distillation in a cyclic still, with the addition of a solvent or carrier, to separate the closely related polycyclic hydrocarbons, fluoranthene and chrysene.

Two equilibrium-type high vacuum stills suitable for 5- to 50-gram samples are described by Zaugg and Shavel (*101*). An external magnetic drive on a horizontal axis is used to rotate a barrel-like surface from which vaporization occurs, and heat is supplied from infrared lamps. A new self-contained vacuum distillation column mounted in a metal shield, and of standardized design for ASTM distillation of lubricating oils and reduced crudes, is available (*5*).

PRECISION DISTILLATION WITH FRACTIONATING APPARATUS

There have been two comprehensive papers on the practical details of precision laboratory fractionation. Winters and Dinerstein (*97*) have described a group of eight columns used for general purposes in a petroleum research laboratory. These are used for 15- to 50-ml. samples and include spinning band, Hypercal, and concentric tube types, each giving a height equivalent to a theoretical plate of about 1 cm. One of the columns in this same laboratory has 300 theoretical plates (*19*). A precisely controlled power supply is stated to be essential to ensure smooth operation, and mixing of fractions must be avoided, as one drop can result in 10% contamination of a fraction. The spinning band apparatus is recommended for vacuum distillation. Adiabaticity is achieved by vacuum jackets and radiation shields, without use of external heaters. The latter are recommended, however, by Cooke and Jameson (*25*), who also describe a group of laboratory fractionating columns for routine analytical distillations. They emphasize the importance of avoiding condensation in the column or reflux divider, and recommend Cannon protruded packing. Several good general discussions of laboratory fractional distillation have appeared in the foreign literature (*18, 24, 28, 49, 69*). The classic work of the Rossini group has appeared in book form (*84*), and a 20-page pamphlet describes the Todd apparatus and gives numerous practical working instructions (*90*).

Several references to continuous laboratory distillation were found (*37, 54, 74, 92*).

AUTOMATIC DISTILLATION AND COLUMN CONTROL ACCESSORIES

There has been little new information reported, as has been indicated by Patterson (*71*) in reviewing automatic analytical operations. Because so many laboratory fractionating columns have for some time been arranged to operate with a minimum of operator attention, it is not surprising to reach a point of diminishing returns. Some foreign articles have appeared (*12, 51*). The idea patented by Bresee (*9*) is probably applicable to laboratory columns. A packed column is provided with a restricted section in which flooding takes place before it occurs generally. Flooding in the restricted section is used as the basis for automatic or manual action to prevent general flooding. A patent was issued on the Podbielniak (*75*) device for a movable thermocouple whose motion spans the zone of condensation at the head of the column.

Wilson (*21*) has described a still head that permits continuous analysis by a measurement of the capacitance of a condenser formed by two concentric metal cylinders surrounding a tube through which condensate flows. Alway and others (*2*) describe a fraction cutter actuated by changes in refractive index or similar properties of the product. Other new fraction-collecting or -cutting devices are characterized by utilization of dielectric constant (*50*), drop counting (*87*), constant volume (*39, 52*), continuous chain belt drive (*35*), siphons (*7*), Teflon plugs (*32*), and other devices (*38, 42, 59, 64, 79, 93, 95*).

Other accessories of interest are a vertical umbrella-type agitator to promote smooth boiling in vacuum distillation (*83*), an automatic safety trap for laboratory steam distillations (*40*), an entrainment catcher (*31*), and a multipurpose apparatus suitable for direct heating, or heating by hot water, steam, or air (*47*).

MECHANICS OF FRACTIONATION

A number of Russian and other eastern European papers have discussed factors affecting theoretical plates (*11, 14–16, 55–57, 86*) and the effect of pressure (*89*). Kuhn, Baertschi, and Thürkauf (*53*) have discussed the relation between various operating variables, Manning and Cannon (*62*) have presented interesting information on channeling, and Rose and Biles (*81*) have described a new method of measuring relative volatilities in an operating column. Fastovskiĭ, Gel'perin, Yoshida, and their coworkers have measured the effectiveness of certain packing materials—namely, gauze plates, porcelain rings, and coke (*33, 36, 100*).

MICRO DISTILLATION, ROTARY APPARATUS, AND ISOTHERMAL DISTILLATION

Javes, Liddell, and Thomas (*48*) have described micro distillation methods for the analysis of petroleum, using about 1-ml. samples and obtaining results in agreement with standard methods, but with three times the experimental error. Fatty oil and surface coating laboratories have also described useful microapparatus. Polymerized methyl eleostearate (*70*) was subjected to distillation analysis by dispersing the sample on a small mass of glass wool suspended from a quartz helix and using a thermostatically controlled aluminum block to control the distillation. The distillate could be collected in sealed ampoules. Various mixtures of monomers, dimers, and polymers were analyzed by Rushman and Simpson (*85*) by placing 100-mg. samples in a small dish on a hot plate located in a zone having a pressure of 10^{-4} mm. of mercury or less. For radioactive materials, Block (*6*) has modified an old apparatus for microfractionation of very small quantities.

Bowden and Cooke (*8*) have separated *o*- and *p*-xylene at low pressure and low throughput rates, using a novel rotary apparatus in which unperforated disks are attached to a rotating shaft. An all-glass rotary film evaporator is described by Volk (*94*).

Childs (*22*) uses isothermal distillation for determining molecular weights and Wust (*98*) has described simple apparatus for isothermal distillation at elevated temperatures, to serve for microdetermination of nitrogen by diffusion of ammonia.

STANDARDIZED AND LOW TEMPERATURE DISTILLATIONS

The ASTM Book of Standards (*3*) contains the latest versions of many tests and standard procedures that involve distillation, and a complete index of all its parts is available. Crozier (*26*) has reviewed the techniques of fractionation in the analysis of petroleum products, from the French point of view. A number of papers have appeared on ASTM, Engler, and related standard distillation tests, apparatus, and procedure (*10, 23, 27, 30, 34, 66–68, 38, 91, 96*). These discuss correlation and prediction, improvement in technique, and apparatus. Preston (*76, 77*) has described improvements in low temperature distillation analysis. There have been further reports on the NGAA analysis program, indicating that errors are due more to human factors than to procedure or apparatus (*20, 73*). Modified apparatus has been described by Deymer, Wustrow, Zdichynec, and Klimicek (*29, 99, 102*).

GENERAL LITERATURE AND BOOKS

A large number of papers have described the use of fractionation or distillation in connection with determination of specific substances. Prominent among these are methods for determination of fluorides, nitrogen or ammonia, crude oils and other petroleum products, oil shale and shale oil, wood, coal and coal byproducts, alcoholic mixtures, fatty acids and derivatives, and metals and their compounds. This general literature has again been indexed and abstracted through 1954, with indexing by substance as well as by method (*82*). The latest version of "Physico-Chemical Methods" by Reilly and Rae (*78*) includes material on both experimental procedure and underlying theory.

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Extraction

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THE writing of this review has been undertaken with frank misgivings about its ultimate worth, partly in view of recent excellent reviews (*73, 105, 106, 147, 148, 150, 152*) and books (*4, 74, 152, 155*) on the general subject of extraction, and partly because the field has become so diffuse. That great strides in the use of extraction for separating and identifying compounds have been made during the past 10 years, no one will deny (*74*). But great strides also have been made with other separating techniques useful in analysis, such as chromatography, paper chromatography, ion exchange chromatography, gas chromatography, and zone electrophoresis. The advances made in all these fields taken together have greatly extended the range of the experimental chemist. Thus it is now possible with substances of molecular weight in the order of a few thousand to separate individual compounds, prove purity, carry out degradations, isolate the degradation products, and determine structure according to the rules of classical organic chemistry with as much or greater reliability than was previously possible with substances in the molecular weight range of a few hundred.

Moreover, the published work in several fields, notably in the polypeptide antibiotics and the hormones of the pituitary, has demonstrated beyond doubt that it is now possible to isolate an unknown natural product, prove purity, and determine its structure without depending on the crystallizability of the substance or its derivatives. Only those who have spent months in previous years patiently trying to induce substances to crystallize, often with complete failure, will fully appreciate the advance that has been made. Extraction has played a very important role in this advance.

It is certain that extraction as a precise separation method would be a much more highly developed and widely used technique than it now is, had not the majority of the research effort concerned with analytical separations during this recent period been devoted to the various forms of chromatography. Only a relatively few laboratories have been concerned with the development of extraction designed for analytical purposes, but hundreds of laboratories throughout the world have devoted part of all of their time to research on some phase of chromatography. In spite of this imbalance, a surprising number of structural studies rightly regarded as epoch-making, such as those with the pituitary hormones, have depended heavily or even been made possible by use of the most highly developed forms of analytical extraction.

FACTORS DETERMINING CHOICE OF SEPARATION TOOL

The major factors which usually determine the choice of a separation tool which is to be used in conjunction with analytical method are:

1. The ease and speed of accomplishment
2. The amount of sample required

3. The general applicability
4. The ultimate over-all selectivity which can be obtained
5. The reproducibility regardless of different solute proportions or presence of extraneous material
6. The number of solutes to be isolated or determined simultaneously

As the over-all use of some form of extraction in analysis has greatly increased during the past few years, it must be assumed that improvements in several or all of these points have been made.

The key to improvement of the first factor is mainly the selectivity of the system. If a single system or a few systems with high selectivity can be found, only a single stage or a few stages will be required. Certainly, a simple extraction is about the easiest analytical separation one could imagine. There has been considerable interest in the development of complexing agents in inorganic analysis (*85*, *102*) for this purpose.

Another approach is through the choice of a spectroscopic method for analysis of the phases, which in itself has considerable selectivity. An intriguing example of this is to be found in a paper by Dean and Lady (*41*). In the determination of iron in nonferrous alloys, the iron was selectively extracted from aqueous acid solution by acetylacetone. The acetylacetone extract was aspirated directly into an oxyacetylene flame and the iron determined by flame spectrophotometry. An increase in over-all selectivity could easily be reached in such an approach without much additional labor by use of countercurrent distribution, employing only a few stages.

In considering the second factor, it should be remembered that extraction is not really an analytical method in itself. It is only capable of bringing about a separation or presenting an environment from which an analytical determination such as a weight determination, a titration, or spectroscopic measurement can be made. Thus the amount of material needed for the over-all process is not necessarily limited by the extraction procedure, but by the sensitivity of the analytical method used in conjunction with it.

Certainly the general applicability of extraction has been greatly improved in the past year or two by the easy availability of hitherto expensive solvents, various combinations of these, and the discovery of new complexing agents such as the fluoro fatty acids (*107*). Many more improvements along this line can be expected in the near future. One scarcely need mention the utility of extraction for separation of the fragile solutes such as the hormones and vitamins found in living tissue.

For highest over-all selectivity in an extraction process, the countercurrent principle must be employed. Here the over-all selectivity will depend on the inherent selectivity of the system, the selectivity of the analytical method used in conjunction with the extraction, and the number of transfers or plates which can be applied. Obviously the highest possible over-all selectivity cannot be reached without some sacrifice of factor 1. Such separations generally require time and labor.

Nonetheless, factor 4 is extremely important and well worth making the sacrifice in many cases, such as in purity studies with biologically active principles of relatively high molecular weight. Thus in the separation of pure β-corticotropin and its enzymatic digestion products for structural studies, Bell and collaborators (*8*) permitted a 200-tube countercurrent distribution apparatus to run by the recycling procedure for long periods of time; in one case until 10,000 transfers had been applied. However, this gave sufficient pure starting material for elucidation of the entire amino acid sequence of a peptide chain containing 37 amino acids. Such a secure foundation for the work is often the deciding point in the success or failure of an expensive research undertaking.

The fractionation studies with insulins form the basis for a concept of ultimate selectivity by correlating it with known chemical structures as mentioned in the last review of this series (*33*). Since that time the complete chemical structures of beef, sheep, and pork insulins have been established by the work of Sanger, Smith, and Kitai (*124*, *125*). An artificial mixture of beef insulin (molecular weight 5733) and pork insulin (molecular weight 5777) (*66*) has recently been separated by countercurrent distribution (*34*) in a system made from 1-butanol, 0.1% acetic acid, and pyridine in the volume proportions of 5, 11, and 3.

This system gives a pH near 6.7 but is not far from the point of mutual miscibility of the phases. Systems of this type with various proportions of pyridine and acetic acid have shown high selectivity with a number of peptides. They offer a range of pH between about 4 to 6.7, which is often optimum from the standpoint of the stability of biological materials. Moreover, the solute can easily be recovered by direct freeze drying.

Bell and coworkers (*8*) in their ACTH studies found an interesting difference in the selectivity of 1-butanol–water mixtures as compared to 2-butanol–water. A similar result was also obtained in the author's laboratory with the bacitracin polypeptides. With separation by countercurrent distribution involving thousands of transfers, a shift in beta value of from 1.0 to 1.1 may mean the difference between success and failure of a difficult separation. Yet such a shift may be caused by a rather small change in the solvent content of the system.

An interesting approach to the problem of selectivity stems from a deliberate attempt to overload the system so that there will be marked solute interaction. An interesting example of this is to be found in the work of Olley (*110*). Two different types of phospholipins could be partially separated in a single run better at high concentration than at lower concentration. The interplay of effects in such an experiment would appear to be too complex to be entirely understandable. This, however, should not prevent practical exploitation. A similar use of the overloading principles has been made in separating tannins (*90*). A more extreme example of this approach for single-stage extraction is to be found in the study of Tavel (*145*). Serum proteins were fractionated by distribution in pairs of partially miscible phases just below the critical point.

The improved separation may result from a peculiar type of isotherm which changes from a positive deviation to a negative deviation as the concentration increases. An isotherm of this general type is shown by nicotine (*60*). Such deviations may be the cause for occasional experimental countercurrent distribution curves which are nearly symmetrical but yet distinctly more narrow than those calculated from the binominal expansion. In the author's experience this type of distribution curve is found only with larger solutes which show strong tendencies to associate. A too narrow curve may give a separation considerably more selective than would be expected on the basis of relative partition ratios.

When such deviations are encountered, reproducibility from experiment to experiment may depend on duplication of the same initial solute concentration and number of transfers. Otherwise reproducibility from run to run, and with different solute proportions, is one of the favorable features of separation by analytical extraction.

No truly objective research worker would expect to solve the more difficult separation problems by use of only one technique. Successive application of different techniques which depend on widely different properties of the molecule is usually much more effective than attempting to wring the highest possible separation from a single approach. Indeed, the outstanding achievements of the past few years have emphasized the need for all possible techniques. As each technique becomes a more highly developed and effective tool, more of a burden is placed on the research worker, who finds he must be thoroughly familiar not only with one approach but also with the many technical details concerned with a number of different fractionation methods.

Even the proper integration of the various techniques becomes a problem in itself. Very good examples of the effective integration of the newer forms of extraction with other techniques are to be found in a number of publications (*8, 71, 90, 101, 113, 114*). This, in itself, can be considered as part of the advance in the field of extraction as well as in other fields. Certainly the use of extraction for a preliminary separation before application of other separation techniques is not new. It was extensively used this way long before it was recognized in itself as being capable of selectivities of the highest order.

In attempting to attain the most effective integration of the various techniques, it is recognized that in many cases several of them could be equally effective, the choice depending on personal preferences and the equipment available in the laboratory. Thus ion exchange chromatography is the most effective tool for the quantitative determination of the known amino acids. But for actual isolation work on new fragments or peptides where ultimate analysis and optical rotations are required, it is not necessarily more effective than countercurrent distribution or zone electrophoresis. Countercurrent distribution has shown itself thus far to be the most effective separation tool for many naturally occurring peptides—e.g., oxytocin, vasopressin, α-corticotropin, β-corticotropin, the tyrocidines, the bacitracins, and the polymyxins.

Although clear generalizations concerning when to use extraction rather than other separation techniques would be highly desirable, the literature at this time does not justify an attempt to form them except on a rather vague basis. In the author's opinion, extraction is the most promising preliminary approach for empirical work in a completely new field. Where applicable, it should be used for the final studies of purity. However, other methods may lead to easier separations once something is known specifically of the properties of the solute of interest.

A good example of this is to be found in the isolation of reserpine, one of the active principles of Indian snake root, which has recently stimulated so much interest in the treatment of mental disease. In the Ciba laboratories (*46*) the active sedative was found in an intractable oleoresin fraction but it caused trouble when further separation was tried. However, countercurrent distribution involving a relatively small number of transfers in a chloroform-methanol-water system separated the resin sharply into two groups of substances, which then could be resolved easily by chromatography. Other examples (*79, 93*) have also appeared of the use of countercurrent distribution in the isolation of this type of alkaloid.

Since the last review of this series (*33*) was written no single major advance in the field can be reported as regards theory, apparatus, or the compounding of systems. However, the work covered in this review shows many improvements for different types of compounds along the lines already suggested in the literature. An attempt to revise the nomenclature and classification of the different types of extraction procedures has been published by Hecker and Allemann (*75*). Many authors may prefer the revised terminology they have proposed; others will not care to change. It seems doubtful if the name "partition chromatography" or "countercurrent distribution" will be changed in the near future in this country. Irrespective of personal preferences, an excellent literature survey of nomenclature has been given.

SYSTEMS

In spite of a much wider use of countercurrent distribution throughout biochemistry and organic chemistry, general rules for the compounding of suitable systems are still badly needed. Some attempts along this line can be mentioned (*72, 105*), but actual determination of partition ratios with a variety of combinations of solvents still remains the most useful approach. Engel and coworkers (*56*) have proposed a nomographic approach to the problem of compounding systems with four components. Rules for compounding three-component systems have been given by Hollingsworth and Taber (*80*).

Several studies can be cited (*29, 33, 74, 105, 115*) which list the determination of partition ratios for a considerable number of organic solutes in various systems with the objective of using these data for identification of the solute. Certain speculations concerning the basis for the ratio, such as hydrogen bonding and structure, have also been made (*3, 116*) and the advantages pointed out of using mixed solvents to improve selectivity. If these data are to be used for countercurrent distribution the partition isotherm must also be considered. This makes a much more complicated state of affairs, as the effect of hydrogen bonding is also a function of concentration. Generalizations are much easier if only gross effects are considered, but for countercurrent distribution suitable factors which shift the K value only slightly e.g., from 1 to 1.1—are extremely important.

The last review of this series (*33*) contained a table with many systems listed for different types of compounds. Such a table could be compiled again in this review, but as additional tables can also be found in two other reviews (*74, 105*), an attempt to treat the literature again in this way is not made. Perhaps a short discussion of research fields where analytical extraction has played an important role will prove of greater interest. Complete coverage of the literature will not be attempted. In most of these fields countercurrent distribution was used only after other fractionation methods had been extensively applied.

Perhaps the most spectacular use of countercurrent distribution has been in the field of the pituitary hormones, both those from the anterior (*2, 8, 9, 28, 82, 99–101, 120*) and posterior (*50, 51, 122*) lobes of the gland. All of these hormones have been found to be polypeptides or proteins of relatively small molecular size. The systems used were of the general type first found useful for insulin (*67*), in which a complexing agent like trichloroacetic acid or *p*-toluenesulfonic acid was used to shift the partition in the direction of the alcohol phase in a butanol-water system. Lysozyme (*32*) has been successfully fractionated this way.

An interesting point about these systems is the striking effect of varying concentrations of the complexing agent. Thus with insulin, the system 2-butanol–0.062% aqueous trichloroacetic acid gave a partition ratio in the right range (0.9), and one which did not vary much with the change of insulin concentration. However, the concentration of trichloroacetic acid was critical. Double the concentration of trichloroacetic acid gave a K of 2.6 but half gave a K of 0.6. The system did not have much capacity because of solubility considerations. A 1% solution of dichloroacetic acid was found to give nearly the same K as 0.062% of trichloroacetic acid but the solubility limit was not reached until considerably more insulin was dissolved.

The compounding of systems which have an organic phase with sufficient polarity to dissolve polar solute such as proteins is one of the problems of applying countercurrent distribution in biochemistry. Perhaps one of the most polar systems yet used is one containing phenol and 2,4,6-trimethylpyridine used in isolating cephalosporin (*1*), a new penicillin more polar than the better known ones.

LIPIDES

A valuable review on the fractionation of lipides by countercurrent extraction has been written by Dutton (*49*). The data obtained by countercurrent distribution have shed new light on the structure of naturally occurring triglycerides. Probably a reasonably "pure" naturally occurring triglyceride has been isolated for the first time in work on the fractionation of the fats of linseed oil (*48*). Discrete bands having 9, 8, 7, etc., double bonds were obtained.

Solvent systems have been worked out for the separation of mono-, di-, and triglycerides (*10*). Diglycerides were identified for the first time as components of the human intestine. Other lipide studies have been concerned with those from fish (*111*) and from serum (*47*). The possibility of separating triglycerides by countercurrent distribution has been discussed by Taber (*143*). The highest possible selectivity is needed for this difficult field.

A short review on the application of countercurrent distribution methods to the phospholipides has been given by Baer (*6*). Precise characterization of many of the phospholipides is very difficult because of their strong tendency to associate and their poor stability. The phosphatidic acids readily undergo hydrolysis even in 85% ethanol. Olley (*110*) followed this hydrolysis by countercurrent distribution.

Studies with soybean phospholipides have included a separation of the color in the "lecithin" fraction (*131*), and further separation of inositoside fractions (*130*).

Countercurrent distribution is an effective way of separating substances that differ in the number of double bonds. This was shown by the separations mentioned above with linseed oil, and was also shown by Klenk and Lindler (*91*) in the separation from brain tissue of C_{22} fatty acids containing one, two, three, and four double bonds.

POLYPEPTIDES

Much work has been done in the field of polypeptides, including isolation of new naturally occurring ones, isolation of fragments following degradation, and purity studies with synthetic material. The most outstanding examples are in the field of the pituitary hormones. These include isolation of probably the first pure ACTH hormone, β-ACTH from beef pituitaries (*8*), α-ACTH from sheep (*100, 101*), the lactogenic hormone (*28*), the melanophore expanding hormone (*9, 99*), and growth hormone (*120*). Countercurrent distribution has been used throughout the structural work on oxytocin and vasopressin and synthetic intermediates for isolation and proof of purity (*51, 52, 122*). The lactogenic hormone probably is the largest molecule (molecular weight 33,000) which has thus far been distributed in a clear-cut way.

The hypertensive polypeptides found in the blood of patients with hypertension is another field which is being rapidly advanced, and countercurrent distribution is playing an important role. Skeggs and coworkers (*133, 134*) have isolated two polypeptides from horse serum after incubation with hog rennin. Bumpus and Page (*21*) have isolated a similar peptide and Kuether and Haney (*95*) have isolated a less stable substance with higher activity.

The studies on the structure of the bacitracin polypeptides (*27, 70, 71, 156, 157*) have revealed some unique structural features on very fragile molecules. These results would have been very difficult to obtain without the extraction techniques. Part of this work involved the separation of pure peptides or derivatives from very complex partial hydrolyzates. Improved selectivity was obtained by distribution of derivatives obtained after distribution of unsubstituted peptide mixtures.

Another very complicated peptide field which has been opened up by countercurrent distribution is that of the streptomyces antibiotics (*15–17, 19, 20, 121*).

A new and interesting member of the penicillin group, cephalosporin, has been isolated (*1*). Countercurrent distribution was found superior to other techniques for isolation of a radioactive penicillin (*31*). Polymyxin B (*69*) has been separated into two peptides which differ in the fatty acid conjugated to the peptide part.

Some of the amino acid sequences in salmine have been determined by partial hydrolysis by enzymes and fractionation by countercurrent distribution (*108*).

Other peptide separations include one from rye grass (*142*), an antibiotic, comirin (*59*), an antibiotic, gramicidin J (*112*), an antibiotic, cinnamycin (*52*), and one from *Fusarium bost* (*24*).

Synthetic higher polypeptides have been separated (*18*). Countercurrent distribution has been found very effective for separating DNP derivatives (*71, 156*) and azobenzene sulfonyl derivatives (*87*) of amino acids and peptides.

ANTIBIOTICS

Separations in this group have included further purification of known types and isolation of new types. Examples are: fummagillin (*62*), neomycin types (*126*), elaiomycin (*68*), aureomycin and Terramycin (*77*), magnamycin (*78, 154*), hygromycin (*104*), methymycin (*45*), levomycin (*26*), and two erythromycins (*119*).

STEROLS

Urinary metabolites of administered corticosterone (*57*) have been isolated. A countercurrent distribution procedure for quantitative-qualitative analysis of urinary corticosteroids (*144*) has been suggested. Hormones of the adrenal cortex have been separated (*25, 132, 153*).

ALKALOIDS

When the development of countercurrent distribution was first begun (*35*) it was with the field of alkaloids in mind because of the possibility of exploiting the high selectivities possible in buffered systems (*36*). In spite of the fact that this was well demonstrated in the synthetic quinoline antimalarial field (*38*), it is only recently that wide use is being made of the method for alkaloids. Recent papers include alkaloids from Rauwolfia (*46, 79, 93*), veratrine (*92*), ergot (*76*), tropane alkaloids (*12*), heliotropium alkaloids (*39*), pilocereine and anhalonidine (*44*), cuauchichicine (*42*), and laurifoline (*43*).

MISCELLANEOUS SEPARATIONS

These include sulfonamides (*14*), bitter principles from hops (*81, 123*), biotin sulfoxide (*160*), pyrrolidine carboxylic acid (*55*), dermatitic compounds from molds (*13*), tromexan (*22*), methyl homologs of 1,2-cyclopentenophenanthrene (*23*), tannins (*89, 90*), colchicine and derivatives (*7*), degradation product of vitamin B_{12} (*94*), azaserine (*61*), pentachloronaphthalene (*11*), and radioactive metabolic products (*146*).

INORGANIC SEPARATIONS

The use of extraction in the field of inorganic separations has come to be a large and very involved subject which cannot be covered adequately in this review. Only a few recent references which give a general idea of the things being emphasized will be cited. It is a field in which much more improvement can be expected in the near future.

In the main the extractions are single stage with emphasis on some type of complexing agent used under carefully controlled conditions in order to achieve selectivity. But when selectivity is not sufficient, attention is being given to some type of countercurrent process such as with the separation of the rare earths (*5, 159*). However, the inherent possibilities have not as yet been exploited to the degree cited in any of the organic or biochemical fields covered in this review.

A very good review of systems and complexing agents to be used in analysis of the precious metals has been given by McBryde (*102*). A number of separations are also listed in other reviews (*72, 85*). Japanese workers have been very active in this field. They have studied the determination of cobalt (*139*), iron (*141*), and mercury and bismuth (*140*) by extraction of an antipyrine complex with ethyl acetate and by extraction of a thiocyanate complex with 2-butanol. The solutions were analyzed spectrophotometrically. Lead, silver, and mercury (*136*), cadmium and arsenic (*137*), tellurium (*64*), and copper and bismuth (*138*) were determined in a similar manner by extraction of the diethyldithiocarbamate complex with benzene or chloroform. Cobalt was also determined as the thiocyanate (*84*). Extraction of the iodides of bismuth and mercury was studied (*65*). An extraction method for separating arsenic and phosphorus (*63*) was given.

The use of extraction for the purification of germanium as the tetrachloride (*109*) and as the tetrabromide (*96*) has been studied.

Beta-diketones are very useful complexing agents. Acetylacetone (*135*) has been studied for extraction of aluminum, gallium, and indium. Fluoro-β-diketones (*83*) are excellent for separating zirconium and hafnium. Separation factors on the order of 13 to 25 were obtained.

Systems made from an aqueous solution of a strong acid and a solution of methyldioctylamine or tribenzylamine are very selective for separating cobalt or zinc (*103*) or niobium and tantalum (*54*). These authors believe an analogy exists between such long-chain or aromatic amines in liquid-liquid extraction and an ion exchange resin.

The system made from tributyl phosphate and hydrochloric acid (*53*) is selective for scandium.

MATHEMATICS

The mathematics for calculating theoretical curves for countercurrent distribution have been treated in a number of places (*30, 74, 155*). A nomograph relating number of plates, per cent of impurity, and values for binary mixtures has been given (*97*). The mathematics involved (*74*) in relating these factors have also been discussed elsewhere.

A series of papers (*30, 117, 127–129*) has given a very thorough treatment of the type of mathematics used in the interpretation and operation of stage continuous columns of the Scheibel type. While this work as it is presented holds considerable more interest for those interested in production than for analysis, it is of great interest for the latter. Compere and Ryland (*30*) have applied the probability concept of "random walk" to double withdrawal. Mathematics for double withdrawal calculations are also given in Hecker's book (*74*). The calculation of deviation from the steady state has been treated by Scheibel (*129*), while holdup and approach to the steady state have been treated by Peppard and Peppard (*117*).

Although no good purpose would be served by pointing out specific papers, there has been a certain amount of abuse of the method of countercurrent distribution for the proof of purity. An experimental curve only slightly broader than a calculated curve cannot be taken in itself as evidence that the preparation is "nearly pure," unless the reason for the spread has been shown by determination of *K*'s across the band to be due to some other cause. A run giving a curve which almost agrees with the theoretical at 100 transfers may clearly reveal two major components at 1000 transfers. This is a matter of the ultimate selectivity of the method.

APPARATUS

In reading the literature of organic chemistry one frequently finds a paragraph in the experimental part in which some small variation of the Kutscher-Steudel or Soxhlet extractor is described. A number of these can be found in fairly recent papers, but those seen by this reviewer were not sufficiently different from variations already published and mentioned in earlier reviews to warrant treatment.

There has been much more interest in trying to improve existing apparatus for bringing about some form of countercurrent extraction. Little has appeared concerning the application of the truly continuous type of extractor in the analytical or small scale preparative field. No doubt this is due to the fact that only a few stages at best are reliably accomplished.

An interesting application of this type of extractor deals with the separation of acids and bases (*158*). The column is so run that there is a pH gradient from the top of the column to the bottom. This sets up a rather complicated state of affairs which exploits the selectivity of buffered systems. The high selectivity of such systems in countercurrent distribution (*36*) was demonstrated more than 10 years ago.

In contrast to the strictly continuous columns, there has been a moderate amount of interest in semicontinuous equipment of the "mixer-settler" type. No doubt this stems from the desire to fractionate larger quantities of material. While there is distinct merit in this as contrasted to countercurrent distribution where kilogram or larger amounts are involved, it should not be assumed that preparative amounts cannot be separated by countercurrent distribution. Although exact details of the apparatus were not described, Patterson and others (*114*) used a 40-tube countercurrent distribution apparatus in the isolation of protogen from 4 tons of liver. Each tube of the apparatus required 1 liter of each phase. An installment in the Squibb Co. contains 24 tubes also accommodating 1 liter in each phase.

With volumes of this size the fundamental pattern could be accomplished with amounts in the order of 1 kg., and by using the alternate withdrawal procedure much more could be processed. All-glass trains with units varying in size from 2 to 1000 ml. are available commercially in this country (H. Post, Maspeth, N. Y.).

Although there has been more interest in the development of extractors of the mixer-settler types, nearly all the actual separations reported in the literature of organic and biochemistry in the past few years have been made by countercurrent distribution, a strictly discontinuous type of extraction. A good example of a separation by a column of the mixer-settler type is to be found in the work of Karr and Scheibel (*86*).

An interesting and new type of mixer-settler is that of Kepes (*88*) in which the heavier phase remains stationary as in countercurrent distribution. A series of partitions in a long glass tube keeps the heavier phases from migrating. The lighter phase is caused to pass up through a small opening in each partition by pressure in such a way as to cause mixing with the heavier phase. A series of such columns can be connected by a tube leading from the top of one to the bottom of another. The effluent phase is collected from the top of the last tube by a fraction collector. The column operates in a way analogous to the "single withdrawal" procedure (*37*) of countercurrent distribution, but as the column is continuous in operation, a "countercurrent distribution" is not achieved. Countercurrent distribution is a strictly discontinuous idealized extraction process operating in such a manner that each step coincides with a stage of the binomial expansion. The simplicity of the Kepes column is attractive.

Other mixer-settlers include one made from separate units cut from a clear plastic (*5*) and clamped together. A stacked series of metal trays clamped together (*58*) on another offers a compact design. Other designs (*40*) for accomplishing mixing-settling have been investigated.

An interesting column of the pulsating Van Dijck type has been described by Werning and others (*159*). It is made from polyethylene in order to permit the use of hydrofluoric acid in the system. The organic phase is methyl isobutyl ketone. This system is favorable for separating tantalum and niobium.

Countercurrent distribution equipment is now made commercially in the United States (H. Post, 6822 60th Road, Maspeth, N. Y.), England (Quickfit & Quartz, Heart of Stone, Staffs), and Germany (Hermann Kuhn, Hospital Strasse 4C, Göttengen). All use a design of tube operating by decantation similar to that first made in this country (*37*). Verzele (*149, 151*) has modified the tube slightly to permit the heavier phase to be moved as well as the lighter phase which would be moved in the opposite direction. This was also possible in the design of Lathe and Ruthven (*98*). The advantage of being able to move either or both phases is in accomplishing alternate or double withdrawal for preparative work. There is no difficulty of accomplishing this in hand-operated trains by the design of tube (*37*) most commonly used. However, a fully automatic train in which center feed could be employed would offer greater advantages, if one were available.

Complete details of none of the three countercurrent distribution separators commercially available are to be found in the literature. The design and arrangement of the tubes are patterned rather closely after that first published by Craig and coworkers (*37*), but the robots and filling devices are different. The one available from Post has a greatly improved filling device and robot compared to the original. This reviewer has not seen the equipment made in England and Germany.

An ingenious new design for the robot has been published by Perry and Weber (*118*). It uses a three-phase motor to withstand the repeated starting, stopping, and reversing required. The excellent step-up switches now commercially available are used to control most of the motions and intervals. This makes for simplicity of design.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Ion Exchange

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IF ONE judges the value of ion exchange techniques to the analytical chemist by the number of published articles and books on the use of ion exchange in analytical chemistry, as well as by the number of commercially available ion exchange resins tailored specifically for the analyst, one must concede that this analytical principle or technique is well accepted by the majority of analytical chemists. This status has been achieved only recently, as it has been only during the past few years that specific procedures and quantitative principles for ion exchange techniques were available to the analyst. The continued development of ion exchange techniques in analytical chemistry must be accompanied by a better understanding on the part of the analytical chemist of the physical chemistry involved and of the nature and properties of the ion exchange resins. Steps in this direction are now evident, judging by the fact that many undergraduate and graduate analytical chemistry courses in many universities and colleges include the subject of ion exchange.

A review of the developments in this field of activity during the past 2 years reveals several important facts. The number of detailed quantitative procedures involving the use of ion exchange resins is gradually increasing. In addition, the availability of ion exchange resins tailored for the analyst has improved considerably and the resins can now be obtained readily from several sources of supply. Simplification of the chromatographic theory as applied to ion exchange columns has resulted in a set of equations less cumbersome, although less rigorous, than those available previously; and these are readily employed by the analytical chemist in developing and modifying procedures.

It is obvious to those familiar with ion exchange in analytical processes that ion exchange techniques function primarily as a means for separating and concentrating various ionic substances and are used where conventional qualitative or quantitative measurements cannot be directly applied to the system in question.

REVIEWS

During the past 2 years, many developments in ion exchange of interest to the analytical chemist have appeared. Several reviews summarize these advances. Deuel (*4A*) reviewed rather extensively the properties of ion exchange resins from a structural standpoint. Kunin (*11A*) outlined progress in the use of ion exchange in a highly condensed but comprehensive article. A special issue of the *Annals of the New York Academy of Science* was devoted to ion exchange studies, particularly in biological systems. In this issue Bregman (*1A*) described the phenomena of cation exchange processes; Craig (*3A*) reported on the factors involved in ion exchange resin synthesis; Ezrin and Cassidy (*5A*) commented on some work on electron exchange polymers prepared from hydroquinone; and Sollner (*14A*) reviewed the properties of ion exchange membranes. Hiester and Phillips (*8A*) summarized many of the more recent developments in ion exchange. Spiegler (*15A*) reviewed ion exchange resins from an electrochemical standpoint, emphasizing the specific conductances and ionic mobilities in resins and membranes. Griessbach (*6A*) summarized the published work to date on the preparation of ion exchange resins having unusual selectivities. A conference on ion exchange technology was held in London in January 1954 (*2A*).

Several reviews were presented which were more specific with respect to analytical problems. Pallaud (*12A*), for example, summarized the use of ion exchange resins in analytical problems; and in Japan, Honda (*9A*) covered much the same ground. The use of resins in microchemistry was reviewed by Kulberg and Lenskaya (*10A*). An excellent collection of papers on chromatography has been issued by the British Medical Society. Several other reviews on chromatography of interest to those employing ion exchange techniques were prepared by Williams (*16A*), Hesse (*7A*), and Ryabchikov (*13A*).

Table I. Selectivity Scale for Monovalent Cations

(Sulfonated styrene-DVB copolymers)

Cation	4% DVB	8% DVB	16% DVB
Li^+	1.00	1.00	1.00
H^+	1.30	1.26	1.45
Na^+	1.49	1.88	2.23
NH_4^+	1.75	2.22	3.07
K^+	2.09	2.63	4.15
Ag^+	4.00	7.36	19.4

THEORY

Several noteworthy advances were made in the theory of ion exchange, and particularly outstanding has been the increase in the amount of experimental data which is now available to test several of the theoretical approaches. Helfferich (*22B*) summarized many of the important basic problems in ion exchange theory, emphasizing the role of ionic hydration in equilibrium studies. Ongaro (*29B*) studied ionic selectivity from an electrochemical point of view. Duncan (*14B*) summarized the thermodynamic properties of ion exchange resins and has calculated the enthalpies and entropies for ion exchange reactions involving the sodium-hydrogen and the barium-hydrogen exchange systems.

Boyd, Soldano, and Bonner (*7B*) determined by means of tracers the diffusion rates in cation exchange resins. Boyd and Soldano (*5B*) extended this study to include a series of sulfonic acid cation exchange resins of varying degrees of cross linking. The self-diffusion constant was found to be dependent upon the ionic charge and was reduced markedly by increasing the degree of cross linking. Activation energies varied from 4700 to 10,000 cal. per mole and were dependent upon the degree of cross linking. The same authors (*6B*) studied the diffusion of water into the exchanger. Bonner and coworkers (*4B*) estimated the activity coefficient within the resin phase by means of an analogy with soluble benzenesulfonic acids.

Soldano, Boyd, and Larson (*35B, 36B, 38B, 39B*) investigated the osmotic properties of a series of resins of varying degrees of cross linking for a large number of ions. The selectivities calculated from the osmotic data were in good agreement with those obtained by other methods. Bonner (*3B*) developed a relative selectivity scale of some monovalent cation exchange reactions for a series of sulfonated cross-linked styrene-divinylbenzene copolymers (see Table I).

Whitcombe, Banchero, and White (*42B*) studied the equilibrium between sodium and potassium chloride and a strongly acidic cation exchange resin, and Wilson (*43B*) determined the effect of ionic strength on silver-sodium exchange equilibrium. Pauley (*30B*) developed a method for the calculation of cation exchange equilibria based upon a consideration of coulombic forces in an exchange system. In this method, the resin is considered as a series of negative point charges randomly distributed and surrounded by cations held at the distance of closest hydrated approach. Free energies for the exchange reactions were calculated by use of classical electrodynamics. Davies and Yeoman (*11B*) derived a method for prediction of cation equilibria based upon an estimate of activity coefficients in the resin phase. Diamond (*12B*) reported equilibrium data for beryllium, calcium, strontium, barium, radium, sodium, rubidium, and cesium cations in acid solutions. Högfeldt (*23B*) extended his study of the silver-hydrogen exchange on a cation exchanger and Davidson and Argersinger (*9B*) collected and correlated equilibrium constants for many cation exchange processes.

Equilibrium studies for anion exchange systems have not been so numerous as those of cation exchange systems; however, some noteworthy contributions have been made. Gregor, Belle, and Marcus (*20B*) determined selectivity coefficients for strongly basic anion exchange resins for univalent anions and Gottlieb and Gregor (*18B*) measured the mean activity coefficients for a variety of materials diffusing into strongly basic anion exchangers. Soldano and Chesnut (*37B*) employed osmotic measurements to estimate the selectivities of strong base anion exchangers for the systems bromide-fluoride, bromide-chloride, and bromide-iodide. Peterson and Gowen (*31B*) presented equilibrium data for the adsorption of aromatic acids on weakly basic anion exchange resins.

The effect of mixed solvents on the volume changes of ion exchange resins for the water-methanol, water-ethanol, water–isopropyl alcohol, and water-dioxane systems was investigated by Gregor, Nobel, and Gottlieb (*19B*). Sundheim, Waxman, and Gregor (*40B*) obtained data for moisture sorption on resins as a function of resin structure. Bafna (*2B*) conducted several ion exchange equilibrium studies in an aqueous-acetone system. Davies and Yeoman (*10B*) illustrated the effect of swelling on equilibrium values for certain cation exchange systems.

Because the rate of exchange is important for processes involving chromatographic separations, a consideration of the developments in this field is most important. Wells (*41B*) reviewed this subject for inorganic ions, and several other excellent papers deal with advances in the mathematics of fixed bed theory. Rosen (*33B*) presented a general numerical solution to the problem of solid diffusion in fixed beds, and Goldstein (*17B*) extended the theory of such processes with a basic review of ion exchange columnal theory. Dickel and Meyer (*13B*) evaluated these processes from the standpoint of temperature effects. Conway, Green, and Reichenberg (*8B*) determined the rates for reactions involving a carboxylic cation exchanger. Freiling (*15B*) found that one could improve upon standard chromatographic techniques by using a gradient elution procedure. Lakshmanan and Lieberman (*28B*) extended this technique to biochemical systems. Glueckauf (*16B*) extended the "theoretical plate" concept for various column separations, and Baddour, Goldstein, and Epstein (*1B*) applied Goldstein's earlier theoretical analysis to the nonequilibrium elution of partially saturated ion exchange beds. Rieman (*32B*) contributed an ion exchange experiment to demonstrate ion exchange chromatography to high school students.

The kinetics of ion exchange reactions between two ion exchange resins has been the subject of an extensive study by Krishnamoorthy (*25B–27B*). This work included a study of the exchange of univalent ions between two different cation exchange resins, between two like anion exchangers, and between mixtures of anion and cation exchangers. Simpson and Wheaton (*34B*) summarized their studies to date on ion exclusion techniques. Gregor (*21B*) and Kressman (*24B*) employed ion exchange membranes to measure ionic activities in solution.

INORGANIC SEPARATIONS

Several interesting analytical procedures are based on the separation of cations by ion exchange and the elimination of interfering cations. The use of cation exchange resin techniques in the separation and analysis of the alkalies and alkaline earths was the subject of many contributions to the technical literature. Okuno, Honda, and Ishimori (*83C*) reported some results on the separation of lithium, sodium, and potassium by a strongly acidic cation exchanger. Ashton and Williams (*2C*) developed an analytical method for the determination of potassium. Sutton and Almy (*114C*) devised a rapid method for the determination of the alkaline components of ash from milk. Samuelson and Sjostrom (*93C*) developed an ion exchange procedure for the determination of alkali metals in the presence of alkaline earths. Rubidium and cesium (*101C*) were concentrated from sea water by ion exchange. Similar separations were reviewed by Rieman (*89C*). A variety of problems involving the determination of alkaline earths were solved using strongly acidic cation exchangers (*1C, 13C, 15C, 19C, 22C, 69C, 71C, 77C*). Lerner and Rieman (*68C*) developed a quantitative separation procedure for the alkaline earths. A method for the separation of barium and strontium was developed by Bovy and Duyckaerts (*14C*) and Hahn and Straub (*44C*). Bertrand and Myers (*10C*) separated ammonium and guanidine salts by cation exchange. Determination of ammonium, amide, and nitrate nitrogen in plant extracts was described by Varner and others (*117C*). Rhodium and iridium separations were accomplished by ion exchange techniques (*8C, 23C*). Separations of zinc from cadmium and magnesium were successfully carried out with the aid of ion exchangers (*5C, 17C, 35C*). Lead was concentrated (*38C*) and dissolved (*84C*) by means of cation exchange.

The use of the high capacity ion exchange resins has become an accepted procedure for concentrating extremely dilute solutions of electrolytes prior to their estimation. This technique has been employed in several hydrological and geochemical field studies. For example, rain water analysis has been facilitated by this technique (*18C, 88C, 116C*), and Nydahl (*82C*) used similar techniques for the analysis of lake waters.

Boiler scale analysis for total solids has been simplified by ion exchange methods (*67C*). Lane, Larson, and Paukey (*64C*) developed a convenient steam purity test. Gabrielson (*31C*) devised an ion exchange procedure for determination of the total metal content in phosphating solutions. Total anion concentration of uranyl solutions was determined by ion exchange by Day and others (*25C*). A method for the separation of ferric iron and aluminum was developed (*55C*). Kadomtzeff (*54C*) studied the diffusion of nickel and cobalt ions in chromatographic separations.

Ion exchange continues to play an important role in the analytical chemistry of transuranic elements, rare earths, and other elements of importance in atomic energy studies. Beryllium, a common element in nuclear reactors, has been concentrated by ion exchange prior to its determination (*90C, 115C*). Scandium (*51C*) was purified from rare earth contamination by cation exchange. Gabrielson (*32C*) removed interfering ions from plating bath solutions prior to determining boric acid. Gallium, indium, and germanium (*60C*) were separated from other metals by ion exchange. The ion exchange separation of the rare earths was improved with the use of sequestering agents as the eluting reagents. Brooksbank and Leddicotte (*16C*) developed extremely sensitive tests for the rare earths in animal tissue. Wish, Freiling, and Bunney (*118C*) employed lactic acid as an eluent for trivalent actinides. Stewart (*111C*) used ion exchange techniques for determination of trace amounts of rare elements of the yttrium group. Similar studies on a macro scale were reported by Spedding and Powell (*104C*). Copper was used as the retaining ion in the elution of rare earths with ammonium ethylenediamine tetraacetate (*108C*). Spedding and Powell (*106C*) discussed the theory of these separations and their group (*105C*) studied the elution of neodymium using citric acid–ammonium citrate solutions. The use of EDTA as a complexing agent for the elution of rare earths from cation exchangers has received considerable attention (*72C, 74C, 107C*). Thorium was determined in the presence of rare earths (*49C, 52C*). Stevenson and others (*110C*) described the separation of the platinum group metals on a cation exchange resin. Zirconium and hafnium (*7C, 66C*) were separated on a cation exchange resin using perchloric acid and nitric acid–citric acid solutions as the eluents. Antimony was determined in the presence of tin by means of an ion exchange procedure (*59C*).

Ion exchange procedures have been utilized to provide rapid methods for the separation and determination of short-lived radioactive materials. These techniques are now standard for the evaluation of transcurium elements obtained by irradiation in nuclear reactors. Glass (*37C*) reported the separation of americium and curium using chelating agents as the eluents. Diamond, Street, and Seaborg (*28C*) examined the exchange properties of the actinides by means of an ion exchange process. Our knowledge of the chemistry of americium-241 and -243 was extended by purification of this elements on a cation exchanger (*27C, 45C*). Elements 99, 100, and 101 were isolated and their existence verified by ion exchange (*3C, 29C, 33C, 34C, 113C*). Studies on uranium chemistry were facilitated by these techniques (*53C, 95C*). Japanese analysts (*58C*) developed a complete ion exchange procedure for the radiochemical analysis of "Bikini ashes" which fell on the ship, *Fukuryn Maru*. Several workers employed ion exchange for a variety of other radiochemical problems (*39C, 96C*).

Contributions were made in the field of chromatographic separations of inorganic elements by Pollard (*86C*), Hiester and others (*48C*), and Carleson (*20C*). Trace metals in petroleum were determined by Sherwood and Chapman (*98C*), and Baticle (*6C*) followed chromatographic separations by means of conductometric methods. Physical variation in ion exchange systems, such as swelling and shrinkage, have been used for analytical purposes (*85C*). New materials have been prepared which will aid in the evaluation of oxidation-reduction reactions (*40C, 103C*). Reactions involving ion exchange resins in liquid ammonia have been examined as possible separation systems (*57C*). Techniques for rapid analysis of iron were reported by Kojima and Kakihana (*61C*).

Lillin (*70C*) reviewed the synthesis of exchange resins which contain groups having selectivity for heavy metals. Metal-amine complexes were studied by Shaw and Bordeaux (*97C*) and Stokes and Walton (*112C*).

Membranes containing ion exchange properties were used in several inorganic systems and their properties measured. Wyllie and Kanaan (*119C, 120C*) reported the basic properties of such materials. Spiegler and Coryell (*109C*) and Schlogl (*94C*) obtained data on their electrical properties. Bergin and Heyn (*9C*) employed such membranes in liquid ammonia systems. Ion exchange resin membranes were also used as electrodes for the determination of ion activity in solution (*99C, 100C*). Salt bridges were constructed using these membranes and these bridges were reported to be superior to conventional salt bridges (*21C*).

Anion exchange resins were studied further for the concentration and separation of numerous metallic anionic complexes. The ability of certain metals to form anionic complexes when in hydrochloric acid solutions has been used to advantage by a number of investigators. Kraus, Nelson, and Smith (*62C, 63C, 79C, 80C, 81C*) examined these reactions for the separation of a large variety of metallic anions including gold, titanium, vanadium, scandium, indium, gallium, thallium, palladium, and platinum. Similar methods were developed by Hague and others (*41C*, *42C*) and applied to the analysis of alloys. Miller and Hunter (*76C*) used such procedures for the determination of zinc in brasses. Hahn, Backer, and Backer (*43C*) and Beukenkamp, Rieman, and Lindenbaum (*11C*) used strongly basic anion exchangers for the separation of phosphates and condensed phosphates. Hall and Johns (*46C*) employed strongly basic anion exchange resins for the separation of technetium from molybdenum, cobalt, and silver. An analytical separation was developed by Meloche and Preuss (*75C*) for the accurate determination of rhenium after an ion exchange separation of that element from molybdenum. Russian workers summarized their studies of a similar problem (*91C*). Sulfate determinations were accomplished by an ion exchange procedure using a barium precipitation (*50C*). Chlorides in detergents were measured after an ion exchange concentration step (*87C*). Langvad (*65C*) attempted to separate chloride isotopes by adsorption on deep beds of strongly basic anion exchange resins. A chromatographic separation of the halides was reported by DeGeiso (*26C*). The platinum group metals were fractionated using anion exchange techniques (*4C*, *12C*, *73C*). The use of complexing agents has proved valuable in a variety of separations. Gillis and others (*36C*) separated niobium and tantalum, using oxalic acid. Similarly, Kakihana (*56C*) detected trace amounts of germanium by adsorption of a germanium hematoxylic complex on a strongly basic porous exchanger. Tin, antimony, and tellurium were separated chromatographically on a strong base anion exchanger using oxalic acid to develop the chromatogram (*102C*). A theory of complex formation was reported by Coryell and Marcus (*24C*). Nelson (*78C*) studied the separation of alkali metals as their EDTA complexes on anion exchangers. Herber and Irvine (*47C*) completed extensive studies on the separation of cobalt, copper, zinc, and gallium using hydrobromic acid as the complexing agent. Citric acid and its salts were also found to be convenient complexing agents for a variety of separations (*30C*, *92C*).

ORGANIC SEPARATIONS

Ion exchange techniques have become standards for the separation of many important biological substances. The analysis of amino acids has reached the point where their separation by cation or anion exchangers has become the accepted procedure throughout the world (*9D*, *11D*, *12D*, *41D*, *42D*, *44D*, *48D*, *59D*). Perhaps the most interesting recent development in this field has been the use of volatile acids as the eluting agents (*23D*). Strongly basic anion exchangers have been used extensively for the analysis of sugars from various natural sources (*1D*, *15D*, *34D*). Complex condensed ring systems, such as ketosteroids (*30D*), have been separated. The chromatographic separations of peptides (*10D*), nucleotides (*35D*), and proteins (*36D*, *56D*) have been reviewed. Porter (*45D*) reported his findings on the chromatography of proteins on a carboxylic cation exchanger. Grubhofer and Schleith (*18D*) concentrated proteins on a carboxylic cation exchanger. Takemoto (*61D*) isolated type A influenza virus by an ion exchange technique.

Various organic acids were reported on weakly and strongly basic anion exchange resins. Derungs and Deuel (*14D*) developed an ion exchange separation of the mono-, di-, tri-, and tetragalacturonic acids. L-Pipecolic acid was isolated from green beans (*71D*) and the chlorogenic acid content of coffee beans was determined (*68D*). A standard ion exchange procedure was developed for the determination of uric acid in fruit (*63D*). Alginic acid was determined after conversion to its free acid form by ion exchange (*58D*). Schenker and Rieman (*49D*) established conditions for the chromatographic separation of malic, tartaric, and citric acids. Berntsson and Samuelson (*5D*) studied the elution of various organic acids of low molecular weight from both strong and weak base exchangers. Organic acids from grass extracts were separated chromatographically on anion exchange resins (*13D*). Ketones and aldehydes were adsorbed on strong base exchangers (*55D*).

Considerable interest has been expressed in the use of ion exchange resins in the analysis of materials of interest to the medical field. Semimicromethods were developed by Achor and Geiling (*2D*) for the determination of morphine. Grant and Hilty (*17D*) separated morphine and codeine using a porous strongly basic anion exchanger. Various alkaloids were isolated and assayed after elution from a carboxylic cation exchanger (*7D*). A report was presented giving details of a comprehensive study of the quantitative elution of morphine from cation exchangers of varying degrees of cross linking (*67D*). Antihistaminics (*27D*) were determined in pharmaceutical preparations and similar analyses of medicinals have been reported in Europe (*64D*, *65D*, *70D*).

Clinicians have found ion exchange resins to be valuable for certain diagnostic tests. Sobotka and Gregor (*57D*) reviewed this subject, and Martin (*37D*) presented an extensive evaluation of the use of ion exchange in medicine. Segal (*52D*) and Sharp, Hazlet, and Shankman (*53D*) described the technique by which a carboxylic exchanger in the quinine form is used to evaluate the acidity of the stomach. Various other indicators have been used in place of the quinine (*51D*). Hofgaard (*24D*) reported the clinical results of this resin technique. Total serum base (*50D*, *66D*) and the composition of blood plasma (*28D*) have been measured rapidly by ion exchange procedures.

Ion exchange resins have been found useful for a variety of miscellaneous separations and procedures which are of general interest. Ion exchange reactions have been performed in solvents other than water. Bodamer and Kunin (*6D*) carried out studies in ethanol, acetone, benzene, petroleum ether, and mineral oil. Sansoni (*47D*), Gemant (*16D*), and Levi (*31D*) reported on results in similar systems. Changes in rate of exchange and volume of exchangers are the variables of considerable interest in nonaqueous systems. Soaps and detergents (*19D*) were examined for fatty acid content after an ion exchange conversion. Ion exchange membranes were employed for studies in milk (*3D*) and various organic electrolytes (*26D*, *32D*). Acid and alkaline values of ash from food were measured after an ion (*29D*) exchange conversion of the salts to the corresponding free acids. Flavins (*69D*) were purified and concentrated with exchangers.

Hormones from the pituitary glands of beef and hogs were fractionated on a carboxylic cation exchanger (*62D*). Nicotinic acid and nicotinamide (*43D*, *60D*) were quantitatively separated. A method for the estimation of choline esters has been developed using a carboxylic cation exchanger (*54D*). A resin has been proposed for the selective retention of sulfhydryl compounds (*38D*). Work was continued on the use of ion exchangers for concentration and purification of cytochrome C (*33D*). Antibodies were purified by means of ion exchange resins which have been linked with antigens (*25D*). Angiotonin (*8D*), a chemical essential for blood formation, has been studied after purification with a carboxylic cation exchanger. Hirs (*22D*) conducted an investigation of chymotrypsinogen using a weakly acidic cation exchanger.

The specific catalytic activity of ion exchange resins has proved to be of considerable interest in organic chemistry; in many instances, their use aids in the separation of relatively pure products from the reaction mixture. Bernhard, Hammett (*4D*), and Helfferich (*20D*, *21D*) reviewed the theory of such catalytic activity. Sugars were hydrolyzed from plant extracts using cation exchange resins (*40D*). Mowery (*39D*) studied the effect of such catalysts on glycoside formation but reported little selectivity when compared as a function of cross linking in the exchanger structure. Riesz and Hammett (*46D*) showed that the catalytic hydrolysis of esters may be accelerated when quaternary ammonium ions are introduced into the reaction mixture.

ION EXCHANGE RESIN ANALYSES AND TECHNIQUES

The routine evaluation of ion exchange resins is important in any analytical process which involves ion exchange resins. Fisher and Kunin (*3E*) have prepared a complete set of test procedures for the capacity determination of all types of ion exchange resins which are available commercially. Austerweil (*1E*) developed a method for estimating the activity of exchangers. Stoch discussed the general evaluation of ion exchangers for water-conditioning applications (*15E*). Rapid methods for the determination of residual capacity were reported by Fuchs and Wagner (*4E*). The use of a titration procedure for the evaluation of resin capacity and basicity was reported by Strobel and Gable (*16E*). Wet combustion has been proposed as a convenient procedure in analysis of residues on the exchanger (*9E*). Several techniques for elution have been reported and are applicable to the evaluation of certain exchange processes (*5E, 6E, 10E, 14E*). Apparatus was described (*2E*) for simultaneously measuring ultraviolet absorption and the radioactivity of substances being eluted from an ion exchanger. Methods were developed for the determination of residual capacity by tracer techniques (*8E*). Gregor, Belle, and Marcus (*7E*) and Pepper, Paisley, and Young (*11E*) described methods for the determination of structural variables in exchange polymers. The effect of pH on capacity was examined for some special cases (*12E*). Radding, Phillips, and Hiester (*13E*) developed analytical methods for use in the evaluation of continuous countercurrent ion exchange processes.

MISCELLANEOUS ANALYTICAL APPLICATIONS OF ION EXCHANGE

Chromatographic separations were reviewed and described in detail by Lederer and Lederer (*7F*). Lister constructed an automatic fraction collector for chromatographic separations (*8F*). A special ion exchange column has been developed for ion exchange laboratory use (*1F*). Some new resins have been synthesized which may be of interest in future work. Sulfonium groups have been introduced into exchange polymers (*4F*). Ferris (*5F*) prepared exchangers with carboxylic and sulfonic groups. A chelating exchanger has been synthesized and may be of interest for inorganic separations (*9F*). Permselective films having anion and cation exchange properties have been described by Bodamer (*2F*). Bray and Reiser (*3F*) developed a method for recycling complexing agents. The manufacture of a strongly basic anion exchanger was reported from Czechoslovakia (*10F*). Amphoteric exchange resins were prepared in Japan (*6F*).

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Nucleonics

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THIS article reviews the publications in nucleonics from 1953 to the present time. It follows, without overlapping, the material presented in the previous review (*167*).

During the past 2 years, the scope of the field has enlarged tremendously. In this country, the change in the Atomic Energy Act in 1954 caused a veritable explosion among industries as they sought to enter the atomic energy field. In Europe, the Atomic Energy Research Establishment at Harwell, England, as well as several other groups has continued to supply tracers in increasing quantities. Several general papers presented recently (*1, 2, 4, 132, 249, 251, 379, 492*) give excellent summaries of the important uses of radioisotopes in analysis in the United States, the United Kingdom, and Russia.

In reviewing publications of such an extensive field, it is difficult to discuss every pertinent paper. It was felt, however, that this type of review should emphasize the widespread use of radioisotopes in analysis today. An effort has therefore been made to include many references illustrating the use of activation, isotope dilution radioassay, and radiotracer methods in analysis. It is hoped that from such a compilation the analyst will discover uses for radioisotopes in his own work, realizing that this technique is not one that is still in the "curiosity" stage, but rather one that has already "arrived."

It was necessary to omit many worth-while papers because of limitations of time and space and the inaccessibility of certain journals. A number of papers dealing with techniques and measurements have also been omitted because they had already appeared in extensive bibliographies of summary papers. Many such summary papers, which were presented at the International Conference on the Peaceful Uses of Atomic Energy held at Geneva (*399*) in August 1955, are included in this review.

The present paper gives the general references at the beginning of each section, followed by specific references to items of more limited application. It has not been possible to cross index all the methods listed in the general papers. Therefore, under each major section, it is important that the general survey papers as well as the specific individual papers be read for a complete picture of progress in the field.

GENERAL

NUCLEAR DATA

Before radioisotopes can be used in any way, their exact characteristics must be known. At present there are two important compilations of nuclear data available. One, by Hollander, Perlman, and Seaborg (*217*), has the advantage that a preferred half life and in many cases a preferred decay scheme are given on the basis of the authors' evaluation of the original literature. Unfortunately, this table was last revised in December 1952. The second compilation is that of Way and the group formerly at the National Bureau of Standards and now at the National Research Council, originally published as an NBS circular with three supplements (*572*). Subsequent data were published in *Nuclear Science Abstracts* (*574*) and cumulated yearly. The National Research Council group has also made available nuclear data cards (*573*) since 1954. These cards are sent out semimonthly for a subscription fee of $20 a year and report all the latest data in the literature. Since 1954 the data given in the quarterly and yearly accumulations of *Nuclear Science Abstracts* have been exact reproductions of the information on these cards.

The National Research Council is in the process of compiling a new table of nuclear data starting with the elements between calcium and zirconium ($Z = 20$ to $Z = 40$). This compilation will report a best evaluated decay scheme and will also list half lives. The first of these new volumes should be out early in 1956, the remainder, in groups of 20 elements, following roughly at 6- or 8-month intervals.

Also available, but much less complete, are the isotope charts. The General Electric chart (*150*), available without cost, proves very helpful, although last revised in November 1952. More recent nuclide charts include that by Bradford (*55*) and the one

Table I. Radioisotopes Used in Analysis (*217*)

Isotope	Type of Decay[a]	Half Life	Energy of Radiation, M.e.v.	
			Particles	Gamma transitions
H^3	β^-	12.46 years	0.0180	No γ
C^{14}	β^-	5568 years	0.155	No γ
Na^{24}	β^-	15.06 hours	1.39	1.37, 275
P^{32}	β^-	14.30 days	1.701	No γ
P^{33}	β^-	25.4 days	0.27	No γ
S^{35}	β^-	87.1 days	0.167	No γ
Cl^{36}	β^-	4.4×10^5 years	0.714	No γ
K^{40}	β^- (89%) EC (11%)	1.2×10^9 years	1.33	1.46
K^{42}	β^-	12.44 hours	3.58 (75%) 2.04 (25%)	1.51 (25%)
Ca^{45}	β^-	152 days	0.254	No γ
Cr^{51}	EC	27.8 days	...	0.32 (8%)
Fe^{55}	EC	2.94 years	...	No γ
Fe^{59}	β^-	45.1 days	0.460 (~50%) 0.257 (~50%)	1.295, 1.097
Co^{60}	β^-	5.27 years	0.306	1.3316, 1.1715
Zn^{65}	EC (97.5%) β^+ (2.5%)	250 days	0.325	1.102
Kr^{85}	β^-	9.4 years	0.695	...
Sr^{90}	β^-	19.9 years	0.61	No γ
Y^{90}	β^-	61 hours	2.18	No γ
Zr^{95}	β^-	65 days	0.371	0.721
Nb^{95}	β^-	35 days	0.160	0.745
Ag^{110}	β^-	270 days	0.087 (~58%) 0.530 (~35%) 2.12 (~3%) 2.86 (~3%)	Several
I^{131}	β^-	8.141 days	0.608 (87.2%) 0.335 (9.3%) 0.250 (2.8%) Others	0.364 (80.9%) 0.637 (9.3%) 0.284 (6.3%) 0.080 (6.3%) Others
Cs^{134}	β^-	2.3 years	0.079 (21%) 0.235 (6%) 0.640 (54%) 0.676 (19%)	Several
Ba^{140}	β^-	12.80 days	0.480 (30%) 1.022 (60%)	0.162, 0.537, 0.304
La^{140}	β^-	40.0 hours	1.32 (70%) 1.67 (20%) 2.26 (10%)	0.49, 0.82, 1.62
Pm^{147}	β^-	2.6 years	0.223	No γ
Tm^{170}	β^-	129 days	0.968 (76%) 0.884 (24%)	0.0841
Au^{198}	β^-	2.69 days	0.963	0.41177
Tl^{204}	β^- (~98%) EC (~2%)	3.5 years	0.765	No γ

[a] EC. Electron capture.

by Pool and Kundu (*432*), which is dated January 6, 1955. Disadvantages of using a chart of this type exclusively include the difficulty in keeping it completely up to date and the tendency to plan experiments on the basis of its limited description of modes of decay.

These charts and tables contain nuclear data for more than 1000 radioactive nuclides, not more than 100 of which are of particular interest to the analyst. Various methods have been devised to increase the usefulness of the data, so that a person can sort out nuclides of particular characteristics by half life and particle energy. Two punched-card systems for radioisotopes (*44, 320*) have been suggested to help in this problem and one set of punched cards is available commercially (*447*). Hallden (*187*) has made a list of useful beta emitters by energy and half life and Lochett and Thomas (*316*) have re-evaluated the half lives of eleven radiotracers of particular interest.

RADIOISOTOPE PRODUCTION

Most isotopes used in analysis are produced in nuclear reactors, although some are made in cyclotrons. Rupp (*466, 467*) has summarized the current information on the large scale production of radioisotopes. Bakker (*19*) has also presented information on this subject, while Martin and others (*334*) have reviewed the problem of radioisotope production rate in a cyclotron.

Three major sources of radioisotopes are the Oak Ridge National Laboratory in the United States, the Chalk River Reactor in Canada, and the Atomic Energy Research Establishment in Harwell, England. The catalogs (*11, 12, 401*) of these laboratories should be consulted for the availability and cost of individual isotopes.

It is also possible to produce usable quantities of tracer activities in a laboratory with a portable neutron source using the Szilard-Chalmers method. Willard (*581*) has reviewed the basic chemical problems involved in nuclear transformations, while Murin and Nefedov (*368*) summarize the enrichment of radioactive elements by this recoil method. They include 151 references to the original literature and conclude that at present concentrated preparations of radioactive copper, gold, lead, phosphorus, vanadium, arsenic, antimony, bismuth, chromium, selenium, uranium, chlorine, manganese, bromine, iodine, iron, cobalt, rhodium, iridium, and platinum can be obtained by this method and that this list will be considerably extended in the near future. Neutron fluxes suitable for this type of work are readily obtainable from portable antimony-beryllium sources (*401*) at a much lower cost than radium-beryllium sources.

REFERENCE MATERIAL

As can be seen from the bibliography, references to applications of nucleonics in analysis are found in many widely separated journals. *Nucleonics* (*383*) has been particularly useful during the past 2 years in pointing out techniques of assay and measurement. In *Nuclear Science Abstracts* (*382*) most of the innovations and research in this field have been abstracted. Unfortunately, the abstracts do not often include articles in which radioactive tracers have been essential to the development of analytical methods, unless the tracers are mentioned in the title or abstract of the article. The *Annual Review of Nuclear Science* (*7*) summarizes recent developments in all fields of nuclear science. The quarterly pamphlet, *Isotopics* (*239*), issued by the Isotopes Division of the U. S. Atomic Energy Commission, carries up-to-date information on uses, cost, and safety considerations for radioisotopes. Recent issues of this pamphlet have carried lists of slide illustrations (*241*) and movies (*242*) available on loan from the Atomic Energy Commission.

Information on the availability of AEC Reports is found in the introductory pages of each issue of *Nuclear Science Abstracts* (*382*). The reports can be found in some 40 repositories in this country and in a number of repositories outside of this country as part of international agreements. Many individual reports will soon also be available on microcards.

A number of books have appeared in the field during the past 2 years. Several chapters in each of the general books by Friedlander and Kennedy (*149*), Whitehouse and Putman (*579*), and Francis, Mulligan, and Wormall (*146*) are devoted to the use of radioactive tracers. Hughes' book on pile neutron research (*222*) as well as books by Sacks (*469*), Hollaender (*216*), and Comar (*85*) devote some space to nucleonics as applied to analysis. Several other general books (*6, 56, 133, 194, 488, 554*) also present some information on this subject.

RADIOISOTOPES AS SOURCES

The uses of radioisotopes can be divided into two major classifications: those dependent upon the radioactivity as a source of ionizing radiation, and those dependent upon the tracer characteristics of radioactive material. The first of these classifications is considered here.

Crompton (*92*) has given an excellent review of the applications of radiation involving penetration or reflection, and other authors (*388, 440*) have reported numerous specific applications. A summary (*175*) comparing the different γ-ray emitters available for use as radioactive sources has been presented. The spectrum of energies available in portable sources can be extended to below 400 k.e.v. by various β-ray excited x-ray sources (*449, 450*). A portable thulium x-ray unit (*555*) has also been described.

TRANSMISSION

Probably one of the first major uses of radioactivity in industry was the thickness gage. Putman (*439*) summarized developments in this field at the Geneva Conference. Additional summary material on current industrial applications of thickness gages is also available (*390, 593*).

These instruments utilize the fact that nuclear radiations are attenuated to a predictable amount by a certain mass of material. Gages use strontium-90, a strong beta-emitting material, for many applications, or carbon-14, emitting a weak beta particle, for special thin requirements. Extension of this principle to gaging even thinner materials with the weak β-rays of promethium-147 (*328*) or alpha particles (*498*) has been suggested.

An extension of the same principle towards measurement of thicker materials has also been suggested—i.e., using x-rays to gage hot steel strips (*535*). Other thickness gages have been reported for specialized problems (*190, 413*) and approximate formulas for describing transmission and absorption of β-rays have been given (*72, 402*).

Several workers have explored the field of nuclear radiation absorptiometry, which promises numerous applications in the future. Nuclear radiations, whether alpha, beta, or gamma, are slowed down in different ways by ionization, scattering, etc., but for each there is a definite dependence upon the atomic number of the elements in the absorbing material. Thus, by proper calibration, it is possible to determine the composition of certain simple mixtures of solids or liquids or gases. While publications in this field are covered more fully in other reviews, several examples are discussed below.

Leboeuf, Miller, and Connally (*297*) at Hanford have described the use of gamma radiation in absorptiometry. These methods have also found considerable use in the oil industry, where Cranston, Matthews, and Evans (*91*) have used x-ray absorption for the rapid determination of sulfur in hydrocarbons. Hughes and Wilczewski (*224*) have made the same type of determination for sulfur in the range 0.05 to 2.7%, using the x-rays from the orbital electron capture of iron-55.

Jacobs and Lewis (*243*) have developed a method for determination of the percentage of hydrogen in liquid hydrocarbon by simultaneously measuring the density and the absorption of Sr^{90}-Y^{90} β-rays of a sample. The weight percentage can be read from a calibration curve and small corrections can be made for the presence of oxygen, nitrogen, sulfur, lead, etc. They report measurements can be made in 5 minutes, with a probable error of 0.02 weight % hydrogen. Apparatus of this type is commercially available from the Central Scientific Co., Chicago, and Hallikainen Instruments, Berkeley, Calif. Smith and Otvos (*510*) discuss in detail an instrument and procedure for the same type of problem. They report determination of the weight per cent of hydrogen with an absolute accuracy of ±0.1%. Applications of the instrument to density measurements are also described.

Deisler, McHenry, and Wilhelm (*108*) have applied the same principle, using the ionization of alpha particles from a polonium source, in analyzing binary and ternary gas mixtures. They report a precision within 0.2 to 0.3 mole % for binary mixtures.

This principle has even been used in a new type of fire alarm system by Pyrene (*77*), utilizing a small radium source. Whenever smoke or any gas of composition other than air enters the detector chamber, the sharp drop in ionization current causes an alarm to be given.

BACK-SCATTERING

A second category of measurements is based on the back-scattering of radiation from matter. It has been known for a long time that the back-scattering of beta particles from a surface shows a marked dependence upon atomic number. Müller (*364, 366*) has observed, however, what appears to be a perfectly general relationship between the back-scattering of beta particles from matter and its composition. He has shown for 32 elements and some 40 compounds that the back-scattering of beta particles is a discontinuous function of the atomic number, with linearity within each period of the periodic system. Unfortunately, only a portion of Müller's work has been published to date, but the remainder should appear soon.

Hine and McCall (*215*) have studied the back-scattering of gamma-rays as a function of the energy of the primary gamma radiation and the material of the back-scatterer. Another report (*441*) describes the use of the back-scattering of weak thulium-170 γ-rays in sorting coal from shale.

MISCELLANEOUS EFFECTS

Several other reports have mentioned novel applications or interferences of radiation with standard analytical techniques. Pannell (*412*) discussed the effect of highly radioactive solutions on glass pH electrodes, while Dewar and Hentz (*111*) described the potentiometric determination of iron by x-ray oxidation. A microcalorimeter has been calibrated (*42*) by use of a radioactive substance having a constant heat output and the Bureau of Standards describes (*329*) a radiation balance for the microcalorimetric comparison of four radium standards. Maton (*335*) has discussed the calorimetric estimation of alpha emitters.

RADIOISOTOPES AS TRACERS

Most of the interest in radioisotopes for analytical chemistry research today involves their use as tracers. The applications cover a spectrum of methods ranging from the determination of minute amounts of isotopes by activation with nuclear particles, to analysis by isotope dilution, the assay and separation of radioactive materials, and the use of radioisotopes as tracers in evaluating or developing analytical procedures. The term radiometric analysis is given a limited definition in this paper and indicates procedures by tracer methods for elements which are not themselves radioactive. It is not used to refer to any and all procedures using tracers, as is commonly done in some of the literature today.

ACTIVATION ANALYSIS

Activation analysis remains perhaps the most spectacular of the applications of nucleonics to analytical chemistry, because of the high sensitivity obtainable in certain optimum cases. Neutron reactions still account for the bulk of the methods, although a few charged particle and photon activations are reported. Two excellent discussions on the present status of activation analysis were given at the Geneva Conference by Smales (*505*) of the United Kingdom and Jakovlev (*244*) of Russia. Each author cites examples from his own recent and unpublished work. Several other general review-type articles (*60, 61, 206, 299, 300, 503*) discuss advantages and disadvantages of this method of analysis.

Plumb and Lewis (*430*) discuss a number of possible errors which can occur in activation analysis. They point out that for accuracy as well as precision one must watch out for errors in activity measurement, incomplete chemical separation, radioactive contamination in carrier, errors in activation due to competing transmutations, flux inhomogeneities, and self-absorption. However, proper techniques can reduce or overcome these sources of error, just as similar problems have been minimized in spectroscopy.

Because activation analysis utilizes the nuclear characteristics of isotopes, there is no correlation between the atomic grouping of elements and their suitability to analysis by this method. Moreover, it is unfair to expect activation analysis to determine a number of elements simultaneously with high sensitivity. Instead, its prime importance appears to be in determining certain trace elements which present difficulties for other analytical techniques.

Sources of Neutrons. Several articles (*220, 221, 387, 396*) describe reactor facilities, both existing and planned, which could be used as a source of neutrons for activation analysis. Others have reported the use of small portable radium-beryllium (*341*) and polonium-beryllium (*338*) sources for specialized problems of this sort. Burrill and Gale (*68*) describe the use of the van de Graaff generator with tritium or beryllium targets to produce neutrons for activation analysis and present a table listing sensitivities attainable with these sources. Jarrett and Berger (*246*) describe the use of very low power reactors with fluxes of about 4×10^7 neutrons per sq. cm. per second for the production of isotopes and for analysis.

In activation analysis, as in many other analytical methods, most results are compared with standards, as relatively large errors may arise in the absolute determination of particle flux, counting geometry and efficiency, etc. If the absolute neutron reaction yield (cross section) is required, there is available a new compilation (*223*) of these values for both thermal and higher energy neutrons. Problems of time and flux perturbation arising in neutron irradiation have been treated by Lewis (*310, 311*).

Sensitivity. The high sensitivity inherent in activation determination of certain elements is certainly its major attraction. An attempt has been made by Meinke (*340*) to compare the sensitivities obtainable by this method with those available from certain other standard techniques. He compared the reported sensitivity values of Leddicotte and Reynolds (*300*) with values reported in the literature for arc and spark spectroscopy, flame spectrometry, colorimetry, and amperometry. Although this comparison gives only order of magnitude values, it can be useful as a starting point in assaying the value of activation analysis for a particular problem.

General tables of sensitivity values for this method (*68, 206, 244, 300, 505*) and values reported for specific isotopes may vary considerably, for the most part because of differences in techniques. Some of the reported sensitivities are based, however, entirely upon calculations. As some writers neglect to include considerations of detection efficiencies in their calculations, the reader is advised to place more faith in sensitivities which have been experimentally determined.

Analysis Schemes. Schemes to determine a whole set of impurities by activation analysis have been developed by a number of groups. Morrison and Cosgrove (*360, 361*) used scintillation spectrometry to minimize the chemical separations required in determining six different impurities in silicon used in transistors. Atchison and Beamer (*10*) describe in detail their techniques for determining nine trace impurities in magnesium. Smales (*504*) includes detailed separation procedures for determining trace impurities in liquid metal coolants. Other schemes include the determination of trace elements in sea water (*27*), reactor cooling water (*321*), and biological material (*186*).

Deuterium, Lithium, Beryllium, and Boron. A method of measuring deuterium using the (γ,n) reaction was reported (*132*). A 1-curie source of sodium-24 permits measurement of a 25-ml. sample of 0.1% deuterium oxide in a 25-ml. sample to ±2% in about 12 minutes (including time for counting background).

Several groups have taken advantage of the large thermal neutron cross section of the (n,α) reaction on lithium-6 to give tritium. Kaplan and Wilzbach (*259*) find that they can determine lithium to within a precision of ±1%, the only significant interference being lithium impurities in the quartz irradiation tubes. Other authors report the determination of lithium in minerals (*426*) and salts (*207*) by neutron bombardment and use of a nuclear emulsion to detect the reaction products.

Beryllium has been determined by the $\mathrm{Be}^9(\alpha,n\gamma)\mathrm{C}^{12}$ reaction by Odeblad and Nati (*403*), although boron, aluminum, magnesium, and certain other elements interfere.

Boron was detected (*339*) in tissues by the $\mathrm{B}^{10}(n,\alpha)\mathrm{Li}^7$ reaction and subsequent autoradiography of the irradiated tissue.

Nitrogen and Oxygen. Süe (*530*) has continued his previous work on activation with charged particles and reports the determination of nitrogen by the (d,n) reaction on nitrogen-14 to give the 2-minute oxygen-15 isotope. He has detected a few micrograms of nitrogen in a metal to within ±20%. The 1.1-minute fluorine-17 is an interference.

Two different activation techniques have been used for the determination of oxygen. Basile and others (*28*) used the (γ,n) reaction with a betatron to produce the 2-minute oxygen-15, and report a sensitivity as low as 0.1% oxygen in aluminum for this method. The authors report that this nondestructive method can be extended to carbon and nitrogen in organic compounds giving the 20-minute carbon-11 and 10-minute nitrogen-13, respectively. Osmond and Smales (*410*) utilized the secondary tritium particles obtained from a neutron bombardment of lithium fluoride to activate oxygen, giving radioactive fluorine-18. They report analyses of samples containing a fraction of 1% of oxygen, to a precision within ±10%, but point out that results are somewhat dependent upon the complete and reproducible intermixing of the lithium fluoride and sample material.

Sodium, Phosphorus, and Chlorine. Plumb and Silverman (*431*) report a precision of better than 1% and a limiting accuracy of 0.0001% in the range of 0.01 to 0.04% for sodium in aluminum alloys. Leddicotte and Reynolds (*301*) have also discussed the determination of the alkali metals by this method, while Smales and Loveridge (*506*) report the determination of sodium in pure lithium metal down to 0.02 p.p.m. Pauly (*416*) describes the estimation of sodium in potassium nitrate by this method.

Schmeiser and Jerchel (*483*) used cyclotron bombardment to determine phosphorus in paper electrophorograms by an (n,p) reaction giving silicon-31. Decay measurements discriminate against interfering activities. Foster and Gaitanis (*143*) determined phosphorus in aluminum and aluminum oxide by activation to phosphorus-32. Phosphorus contents of 0.001 to 0.0001% were determined to ±5% and a practical sensitivity of $5 \times 10^{-7}\%$ is reported possible.

Another group (*22*) reports the determination of chlorine in luminescent zinc sulfide crystals to a limit of 5×10^{-6} gram per gram of zinc sulfide with a precision within ±10%. This, they say, is five times better than the precision of the same determination by a nephelometric method.

Argon, Cobalt, Nickel, and Copper. Another group (*355*) has applied neutron activation to potassium mineral dating. It determines the argon-40 content of potassium minerals by activation to the radioactive argon-41 and reports measurement of as little as 3×10^{-8} gram of argon-40. It also cites the possibility of using the (n,α) reaction on radiogenic calcium-40 to give radioactive argon-37 in these studies.

Smales and Wiseman (*508*) describe analyses of deep-sea sediments for cobalt, nickel, and copper by this method. Szekely (*536*) determined traces of copper in germanium and reports a sensitivity of $10^{-4}\,\gamma$. A chemical separation of the copper was required. Copper has also been determined in cellulose esters (*434*) by activation analysis.

Arsenic, Technetium, and Silver. Arsenic has been determined in silicon (*245*) by James and Richards with a chemical yield of 80 to 90% and a sensitivity of 0.0003 p.p.m. on a 1-gram sample.

Alperovitch and Miller (*392*) used activation analysis of ores in looking for technetium-98 in nature.

Berlman (*33*) developed a technique for the determination of film exposure by activation of silver.

Barium, Strontium, Cesium, and Rubidium. The estimation of trace amounts of barium or strontium in biological materials can be determined by activation, with no interference from a large excess of calcium (*197*). When chemical separations are used, a precision within ±5% and a sensitivity of 1 to 50 γ is obtained for either element.

Smales and Salmon (*507*) present an excellent discussion of the determination of small amounts of rubidium and cesium in sea water and related materials of geochemical interest. The method and applications are described in detail. Sensitivities of 10^{-6} to 10^{-8} gram are reported for both elements.

Rare Earths and Tantalum. Phillips and Cornish (*424*) discuss the determination of dysprosium in "spec pure" holmium oxide. No chemical separations were made, but aqueous dilutions led to values of 11.71 ± 0.09% by liquid counting and 11.66 ± 0.16% by solid counting. A comparison (*342*) of activation analysis with spectrophotometric procedures has been made for several rare earth elements of high cross section.

Several authors (*38, 278, 350*) discuss the analysis for tantalum in niobium ores and in stainless steels by this method.

Thorium and Uranium. Jenkins (*247*) describes in detail a procedure for the determination of small quantities of thorium by the activation method and compares it with methods using thorium tracer or detecting the natural activities. Leddicotte and Mahlman (*298*) also discussed this determination in their Geneva paper.

A method for the determination of uranium-235 in a mixture of naturally occurring uranium isotopes has been reported (*495, 496*) to give a precision within ±0.44%. Two other excellent papers (*325, 502*) discuss in detail the determination of very small amounts of uranium in rocks and minerals, while a third paper (*526*) describes the determination of uranium in sea water. In this ingenious procedure, uranium was first extracted with dibutyl hydrogen phosphate in carbon tetrachloride and evaporated to dryness. The residue was then subjected to a neutron flux, and fissions of uranium-235 were counted.

NEUTRON ABSORPTIOMETRY

It is possible to utilize the absorption of neutrons for analysis just as the absorption of light, etc., is used in other methods.

Little activity has occurred in this field during the past 2 years, possibly because of the lack of good monoenergetic neutron sources at different energies. Further work on the method of boron determination has been reported (*188*) using a polonium-beryllium neutron source. By this method 0.1% boron in solution can be determined with a precision within ±3%.

ISOTOPE DILUTION

This method is of particular importance in analyzing complicated inorganic or organic mixtures or residues where quantitative separation of the desired material is difficult, time-consuming, or impossible. A measured amount of the pure constituent of known specific activity (counts per minute per gram) is added to the unknown mixture in solution. After thorough mixing to ensure equilibrium, a pure sample of the desired constituent is separated and counted. By comparing the new specific activity with the specific activity of the pure constituent added, it is possible to determine the amount of that material in the mixture. In effect, the radioactive materials are measuring the yield of the separation procedure.

Potentially this is a powerful tool for the analyst. While the separation need not be quantitative, it must give a pure sample, nor may there be any isotopic exchange to reduce the specific activity of the labeled material. Now that so many organic compounds labeled with carbon-14 and tritium are available (*240, 378*), the method should find even wider applicability.

Pinajian and coworkers (*429*) have given an excellent review of the history of this method and list 78 references to the original literature in a survey of the field. Christian and Pinajian (*79*) describe three procedures of analysis—the direct isotope dilution analysis, the inverse isotope dilution analysis, and the double isotope dilution analysis—and discuss the limitations of each. Equations used in the calculations for each of these methods are given.

Quimby, Mabis, and Lampe (*442*) describe in detail their use of phosphorus-32 to determine pyrophosphate and triphosphate in the presence of other phosphates. They report no systematic bias in the analysis for pyrophosphate and a maximum of ±2% for the triphosphate; standard deviations of ±1% for the $Na_4P_2O_7$ and 1.5% for the $Na_5P_3O_{10}$ were obtained. Haywood (*202*) has also discussed the application of this method to the determination of condensed inorganic phosphates. Ikeda and Kanbara (*232*) used this technique in determining elementary sulfur in acetone extracts of vulcanized rubber, while Tarasova, Kaplunov, and Dogadkin (*538*) used sulfur-35 for the study and control of the vulcanization process.

Ashton and Foster (*9*) determined benzylpenicillin in fermentation liquors using the carbon-14–labeled form. By paying considerable attention to detail they were able to obtain a standard error of ±1%. Their method is based upon the determination of a phenylacetyl grouping and thus is complicated by the presence of other phenylacetyl derivatives in the sample. Gordon and coworkers (*171*) have developed an assay method for penicillins in broth using sulfur-35–labeled penicillin. No degradation of the penicillin is required and penicilloic acid does not interfere. They report an error of ±5.9% at the 95% confidence level for their determinations.

A determination of the gamma isomer in crude benzene hexachloride by the use of carbon-14–tagged material was reported by Hill and associates (*212*). They used a methane proportional flow counter and thick barium carbonate samples to give reproducibilities within ±3%. Craig, Tryon, and Brown (*90*) made the same type of analysis using chlorine-36, resulting in a standard deviation of ±0.2% in gamma isomer content for samples containing from 1 to 50% of the isomer.

Sorensen (*514*) reports the determination of hydroxy and amino compounds by a chlorine-36 isotope dilution method, in which the compound to be analyzed is first quantitatively converted to a chlorine-containing derivative. He also uses (*513*) chlorine-36 to determine 2,4-D and other weed killers of agricultural interest.

Sowden and Spriggs (*516*) describe determination of D-glucose and gentiobiose in "hydrol" using carbon-14–labeled sugars. Burtle and Ryan (*69*) used carbon-14 in an isotope dilution procedure to detect small amounts of diethyl ether in large amounts of ester. It is reported (*391*) that the Hercules Powder Co. uses isotopic dilution in analysis of pentaerythritol.

It is possible to assay for vitamin B_{12} in complex mixtures ranging from fermentation products to vitamin capsules by isotope dilution using the vitamin labeled with radioactive cobalt-60. Bacher, Boley, and Shonk (*15*) report for this method a standard deviation of ±4.3% for samples having as low a concentration as 0.1 γ per ml. and a total weight of 0.1 mg. Numerof, Sassaman, Rodgers, and Schaefer (*400*) describe a procedure for the assay of vitamin D using radioactive phosphorus.

Reineke (*451*) used radioactive iodine to determine the thyroxine content of thyroactive iodinated proteins by isotope dilution. A value was obtained that was one third as large as that indicated by the unreliable 1-butanol extraction procedure.

Municio (*367*) has compared isotope dilution with other methods used in the quantitative determination of amino acids by paper chromatography.

RADIOMETRIC ANALYSIS

This method of analysis has remained of only passing interest to date. Many who explored its uses developed methods which had few of the advantages and some of the disadvantages of methods already in existence. A few definite contributions have been made during the past 2 years, however.

Determinations of phosphorus and arsenic (as phosphate and arsenate) by precipitation with radioactive silver as Ag_2TlPO_4 and Ag_2TlAsO_4 have been studied (*173*). The determination of small amounts of thallium by precipitation of the thallium with hexamine cobaltic trichloride labeled with cobalt-60 is also reported (*236*). Two groups (*238, 493*) independently report the determination of microgram amounts of potassium with cobaltinitrite labeled with cobalt-60.

An ingenious method for the quantitative paper chromatography of traces of metals was reported by van Erkelens (*561*). The paper chromatogram was exposed to hydrogen sulfide labeled with sulfur-35. The method shows considerable promise, because it can be applied to biological materials, is cheap, and is reported to have a sensitivity comparable to that of spectrography. Schayer and coworkers (*481*) have determined histamine over a wide range of concentration by converting it to an iodine-131–labeled "pipsyl chloride." They report the reaction is very specific for histamine, with no known tissue constituents interfering.

ASSAY OF RADIOISOTOPES

In any experiment utilizing the tracer potentialities of radioactive materials it is necessary to have techniques available to assay the amount of radioactivity in the end products. Assay methods including both sample preparation and measurement techniques are described in this section. References dealing only with radiochemical separations appear in a later section.

The problems of gross radioassay of such diverse items as industrial wastes (*208, 494*), excretions (*322*), and fish tissue (*289*) have been discussed in detail, and an elaborate report has been written (*394*) on the analysis of the radioactive dust that fell on the Japanese fishing boat after the nuclear detonation in the Pacific. Several new assay techniques and pieces of equipment for sample preparation have been reported (*198, 465, 489*).

In many laboratories the Geiger counter is gradually giving way to the proportional and scintillation counter for assay work. Nevertheless, a number of articles, at least a dozen in *Nucleonics*

(*383*) alone, have discussed the characteristics of Geiger counters (*499*). Discussion of the scintillation counter and spectrometer is reserved for a later section.

Tritium. Although this isotope has found considerable use in tracer studies, its low energy has deterred many because of the self-absorption inherent in its measurement. General problems of tritium research were described by Brown and coworkers (*63*) in their paper at the Geneva conference. Other general references include a book by Glascock (*160*) and a detailed review and bibliography of tritium techniques and applications by Thompson (*545*). Johnston (*250*) presents an excellent discussion of problems in low-level counting and the future of isotopic tracers. His bibliography gives 57 references to pertinent material.

Jenkins (*248*) uses a windowless flow counter and bases his method for estimating tritium content on measuring the activity of solid ammonium chloride made radioactive by exchange with tritiated water. The method is rapid but is limited at present to an accuracy within ±15%. Wing and Johnston (*586*) report a reproducible method whereby tritiated water is converted to acetylene with calcium carbide and the acetylene determined by Geiger counting. Libby (*312*) describes the development of sensitive radiation detection techniques for tritium, and Beischer (*30*) discusses the absolute beta measurement of tritium mono-layers.

In tritium assay, as in any radioactivity assay, there are two major problems: to obtain the radioactive material from the sample in a usable form and to count the material under optimum conditions. Three different methods are suggested for the preparation of gas for assay of tritium in organic compounds. Viallard and others (*564*) use a semimicro combustion train and report a high sensitivity for an analysis requiring only 0.01 to 0.02 gram of sample. Payne and Done (*417*) use a combustion bomb method for the preparation of the gas, while Wilzbach, Kaplan, and Brown (*583*) report using a zinc fusion method.

To counteract the self-absorption of the weak tritium beta particles, Hayes and Gould (*201*) have counted tritium-labeled water and organic compounds with liquid scintillators, while Wilzbach, Van Dyken, and Kaplan (*585*) and Wilson (*582*) describe the use of ionization chambers for the measurement. McClelland and others (*322*) describe in detail a procedure for the tritium assay of urine and water.

Carbon-14. More references to this radioisotope appear in the chemical literature than to any other because of its usefulness in tracing organic reactions. In the assay of organic samples, two methods of decomposition have been used, wet combustion and dry combustion.

In his Fisher Award Lecture Van Slyke (*562*) has given an excellent review of wet carbon combustion and its applications. Other papers (*17, 36, 261, 262, 421, 576*) report modifications in the basic wet-combustion technique. Burr (*66*) sweeps the carbon dioxide formed in the wet combustion directly into an ion chamber for measurement. An electric heater has been designed for the Van Slyke combustion apparatus (*189*). Collins and Ropp (*84*) studied the accuracy obtainable from the Van Slyke wet combustion and radioassay of carbon-14–labeled compounds using an ion chamber and a vibrating reed electrometer. By carefully controlling conditions, they found the relative specific activity of a carbon-14 compound could be measured to within about ±0.5%.

Use of a high frequency induction furnace in the determination of radiocarbon is reported (*157*) to be fast and reliable. Wilzbach and Sykes (*584*) describe a simplified dry-combustion technique in which organic compounds are heated in a tube with copper oxide. The procedure is similar to the zinc fusion technique for tritium. Of probable interest to many is a method providing for the simultaneous determination of total carbon and carbon-14 activity in compounds for which wet-combustion procedures for total carbon are not adequate (*154*).

A number of articles have been written on three of the instruments used for the measurement of carbon-14: the ionization chamber, the proportional counter, and the liquid scintillation counter. Brownell and Lockhart (*64*) describe in detail the procedures required for precise ionization chamber measurements. Raaen and Ropp (*444*) report a precision within ±0.3% at the 95% confidence level for the radioassay of benzoic acids, using a Van Slyke wet combustion in conjunction with an ionization chamber and a vibrating reed electrometer, if all operations are performed in one day. Baker, Tolbert, and Marcus (*16*) have assayed breath carbon-14 in ionization chambers.

Several groups describe (*58, 484*) techniques for using proportional counters for assay of radiocarbon. Karnovsky and others (*260*) compare activities of several organic compounds counted as such or as barium carbonate after combustion. They found that the relevant correction factors were much smaller in a flow proportional counter than corresponding factors reported in the literature for end-window Geiger counters. Other articles also discuss this self-absorption correction (*166*) and methods for obtaining evenly distributed counting plates (*67*). Henson (*204*) eliminates the self-absorption problem by counting carbon-14 as gaseous carbon dioxide in a special Geiger counter.

The liquid scintillation counting of natural radiocarbon was described in detail by Hayes, Anderson, and Arnold (*199*) in their paper at the Geneva conference. Twenty-five pertinent references to the current literature are given. Two other groups (*13, 114*) also have published papers on the use of liquid scintillators in carbon-14 research.

An interesting aspect of carbon-14 work that is equally applicable to work with other radioisotopes is the self-radiolysis and decomposition of carbon-14 compounds upon standing. Two groups (*308, 566*) have studied this effect.

Tolbert, Garden, and Adams describe (*546*) certain special handling equipment used for carbon-14 work.

Phosphorus-32. Hahn and Anderson (*180*) discuss radiochemical methods for the determination of this isotope. Loevinger and Feitelberg (*318*) assay phosphorus-32 with a well-type scintillation counter by counting the bremsstrahlen resulting from absorption of the beta rays in the crystal mounting. Dawson (*103*) measures radiophosphorus in small samples of tissue. The sample is digested and subjected to two-dimensional chromatography and the active spots are eluted from the paper and counted.

One problem inherent in work with phosphorus-32 is the presence in the stock solution of small amounts of the weak beta-emitting phosphorus-33 resulting from second-order neutron capture. Present techniques assay the phosphorus solution for phosphorus-33 by differential absorption curves or spectrometry, but Mayr (*337*) reports the use of nuclear emulsions 200 microns thick to determine this ratio. The emulsion includes almost all the tracks of phosphorus-33 while recording only a small portion of the tracks of phosphorus-32. Rosen and Davis (*460*) describe the absolute beta counting of gases containing phosphorus-32 with an end-window Geiger counter.

Sulfur-35 and Chlorine-36. Two papers (*231, 343*) include methods for the quantitative determination of sulfur-35 in complex organic mixtures. Self-absorption corrections (*343*) are reported for a number of sulfur-containing mixtures. A simple technique for counting milligram samples of protein labeled with carbon-14 or sulfur-35 has been published (*156*), as well as a procedure for digestion and radioassay of animal tissues containing sulfur-35 (*76*). Walser, Reid, and Seldin (*569*) have counted radiosulfur in liquid samples by a method that has special application to the determination of sulfur-35 in excretions following an injection of labeled sulfate.

Sorensen (*515*) used four different organic compounds to investigate the reproducibility of mounting solid samples of chlorine-36 compounds for radioactivity measurement. Kahn and coworkers (*255*) describe assay procedures suitable for chlorine-36 and report that both mercurous and silver chlorides are suitable precipitates for counting. The advantages of solid versus liquid counting are discussed.

Sodium-24, Potassium-40,42, and Calcium-45. Suttle and Libby (*532*) discuss the application of the absolute assay of beta radioactivity in thick samples to the determination of potassium in naturally radioactive potassium salts. Dresia (*120*) uses a Geiger counter to determine potassium in solution with a precision of ±2–3% at a lower concentration limit of 20 mg. per ml. With a special Geiger counter, Scheel (*482*) rapidly determines potassium and potassium salts with an accuracy comparable to the normal gravimetric method. The procedure is adaptable to use in the laboratory or a mine. In the biological field, Orvis and Albert (*406*) have been successful in the bioassay of solutions of aldosterone and related steroids by gamma counting of sodium-24 and potassium-42.

A special technique for determining the specific activity of calcium-45 by use of a collapsible centrifuge tube has been developed (*172*).

Chromium-51, Iron-55,59, Cobalt-60, and Zinc-65. Several authors (*78, 273, 422, 448*) have recorded methods for assaying the isotopes of iron in biological material. Two groups (*448, 527*) have assayed iron-55 and iron-59 simultaneously in mixed preparations. Cheong and coworkers (*78*) discuss problems of assay of iron-55 in multiple tracer studies with high activities of silver-110, while Lough and Hertsch (*319*) describe procedures for estimating the composition of iron-59–chromium-51 mixtures.

Ballentine and Burford (*21*) discuss in detail the radiochemical assay of cobalt-60 in biological materials. In their procedure, which has given a standard deviation of ±3% in over a thousand determinations, samples are wet-ashed, separated as cobaltic hydroxide, electroplated as cobalt metal from a fluoborate buffer, and counted in a special flow proportional counter.

Banks and coworkers (*23, 24*) determine zinc-65 in biological tissues with Geiger and scintillation counting techniques. Wet combustion, extraction with dithizone, or homogenization has been used to prepare the sample for counting.

Krypton-85, Zirconium-95, and Silver-110. Reynolds (*452*) reports techniques for counting radiokrypton, giving detailed diagrams of vacuum line handling and counter system, while Busey (*70*) describes a system of volumetric microanalysis for reactor off-gases. Hahn and Skonieczny (*182*) have determined radioactive zirconium in fission products, using mandelic acid to precipitate zirconium carrier.

Cheong, Perri, and Sharpe (*78*) assay radiosilver in biological samples by scintillation counting techniques.

Iodine-131 and Astatine-211. Weisburger and Lipner (*575*) have carefully evaluated the different counting systems available for the laboratory determination of iodine-131. They conclude that the windowless counter is about equal in efficiency to the scintillation well counter, but that ease of preparation heavily favors the well counter. Brown and Jackson (*62*) describe a technique for the estimation of the radioactive components of plasma after the administration of iodine-131.

Durbin, Hamilton, and Parrott (*123*) have developed a procedure for the codetermination of iodine-131 and astatine-211 in tissue by digesting with chromic acid, reducing with oxalic acid and distilling.

Cesium-134, Strontium-90, Barium-140, and Gold-198. Hahn and Backer (*181*) determined radioactive cesium in fission products by precipitation with dipicrylamine or periodic acid. Determination of radioactive strontium and barium in water has also been recorded (*183*) with sensitivities of about 4×10^{-8} and 10^{-7} μc. per ml., respectively, and a precision of about 10%.

Weiss, Steers, and Bollinger (*577*) report a novel technique for the determination of radioactive gold in large amounts of biological tissue, which depends upon the recovery of the gold from hydrolyzed tissue with activated charcoal. Solutions of from 0.2 to 1000 μc. of gold-198 gave 96 to 107% recovery.

Polonium-210, Emanation, and Actinium-227. Scott and Stannard (*487*) investigated wet and dry oxidation procedures for polonium-210 in biological materials. This same isotope is rapidly determined (*322, 456*) in urine by electrochemical deposition on a nickel disk from acid solution with a yield of around 98%.

Element-86 (emanation, radon, thoron, etc.) has been determined in mineral waters (*291, 292*), in the air (*192, 549*), in breath (*226*), and in food (*497*). A scintillation counter for the assay of radon gas has been described (*559*).

A procedure for the separation of actinium and its daughters by means of lead sulfate from urine solutions has been recommended (*459*).

Radium, Thorium, and Uranium. Wamser and others (*571*) have perfected a rapid preparation for radioassay of residues containing radium. Kirby (*268*) discusses the determination of radium by alpha counting.

Three papers (*234, 427, 433*) discuss procedures for the determination of ionium (thorium-230) and other thorium isotopes in sea sediments, while other references (*5, 94*) describe methods for the determination of mesothorium in thorium-containing materials. Rosholt (*462*) presents a quantitative method for the determination of the major sources of natural radioactivity in ores and minerals.

There has been a great deal of interest in the determination of uranium and its daughters in radioactive ores and solutions (*37, 59, 126, 147, 280, 411, 457, 529, 553*). One group reports (*580*) an electrodeposition method for the determination of uranium in urine. A radiochemical method for the determination of uranium-235 in uranium is also described (*106*).

SEPARATION AND PREPARATION OF RADIOISOTOPES

These papers differ from those in the previous section in that their primary objective is the separation of one radioactive material from other radioactive materials. Several new compilations of radiochemical procedures have become available during the past 2 years. The compilation collected (*274*) and revised (*275*) by Kleinberg and others describes radiochemical procedures used at Los Alamos; that by Lindner (*314*), 20 procedures used at the University of California Radiation Laboratory (Livermore); and that by Finston (*137*), a dozen or more procedures submitted to the clearinghouse supported by the National Research Council. Each of the above procedures is described in detail. Kahn (*253*) has given limited distribution to an extensive compilation abstracting unclassified radiochemical methods for most of the elements.

Other general descriptions and summaries of radiochemical procedures can be found in the articles in *Annual Review of Nuclear Science* by Stevenson and Hicks (*524*) on separation techniques used in radiochemistry (with 140 references), Glendenin and Steinberg (*162*) on fission radiochemistry (with 62 references), and Finston and Miskel (*138*) on assorted radiochemical procedures. Bruce (*65*) has summarized the solvent extraction chemistry of the fission products, while several other articles (*74, 479, 491*) discuss the separation of several radioisotopes from one particular material. The carrier-free separation of a number of isotopes is also described (*302, 475*). Cathers (*75*) has discussed problems of radiation damage to radiochemical processing reagents.

Hydrogen to Zinc. A separation of mixtures of tritium and hydrogen is discussed (*122*). Mellet, Daudel, and Muxart (*344*) report the microchemical preparation of beryllium-7, while other authors (*46, 293*) describe separation procedures for organic compounds labeled with carbon-14. Bernstein and Katz (*34*) give detailed description of the preparation and properties of fluorine-18.

Arrol (*8*) describes the apparatus required for large scale production of phosphorus-32, and another paper (*475*) recommends the carrier-free extraction of this same isotope into boiling water from pile-irradiated sulfur. Separation of carrier-free scandium from a calcium target is reported by Duval and Kurbatov (*124*). The scandium is adsorbed on filter paper while the more concentrated calcium is unaffected.

Harbottle and Maddock (*191*) have prepared chromium-51 of high specific activity by the Szilard-Chalmers reaction on potassium dichromate. Dick and Kurbatov (*112*) describe a carrier-free separation of cobalt-56, -57, -58 from a manganese target by extraction of the cobalt with 1-nitroso-2-naphthol. Mahlman, Leddicotte, and Moore (*326*) separate cobalt by methyl dioctylamine extraction. The carrier-free separation of zinc from cyclotron-irradiated copper by elution from a cellulose column with a 1-butanol–hydrochloric acid mixture is reported by Tupper and Watts (*550*).

Arsenic to Silver. Green and Kafalas (*176*) report the preparation of carrier-free arsenic-74 from a germanium cyclotron target by extraction with benzene from a concentrated hydrochloric acid solution. Laurent and Simonnin (*296*) prepare arsenic-76 by the Szilard-Chalmers reaction on cacodylic acid.

Mizzan (*352*) uses yttrium hydroxide scavenging and oxalate precipitation to determine strontium in fission products, while Wray (*591*) discusses a method for control of strontium-90 purification by cation exchange. Turk (*551*) introduces a modified strontium procedure involving several iron hydroxide scavenges and precipitation as the carbonate. Salutsky and Kirby (*472*) obtain a continuous supply of carrier-free yttrium-90 by a rapid method involving precipitation of the strontium-90 parent as the nitrate. Mizzan (*353*) reports an improved procedure for yttrium in long-lived fission product solutions. Moore (*356*) separates niobium from protactinium by liquid-liquid extraction into diisobutylcarbinol from dilute hydrofluoric-sulfuric acid solution. The separation by ion exchange of technetium from other materials in a neutron bombardment of molybdenum has been reported by Hall and Johns (*185*). Two other studies on the isolation of technetium have also appeared (*351, 548*), the latter using extraction of tetraphenylarsonium pertectnate into chloroform.

A new type of procedure for the isolation of radioactive silver has been reported by Sunderman and Meinke (*531*). This method depends upon the rapid isotopic exchange between silver ion in solution and a silver chloride precipitate. Quantitative yields can be obtained in a few minutes with high decontaminations from most elements. Rouser and Hahn (*464*) outline a method for the separation of the radioactivities of silver and palladium, and Griess and Rogers (*177*) describe a procedure for the selective electrodeposition of silver from a solution of palladium.

Tin to Thallium. Tin, antimony, and tellurium have been separated by anion exchange (*509*). The widespread use of iodine in medical tracer and therapeutic work is evidenced by the number of reports of separations for both iodine-131 and -132. The separation of iodine-131 from tellurium dioxide by dry distillation (*475, 539, 540*) and the production of iodine-132 (*519*) have been reported in detail. A radiochemical iodine procedure involving distillation and extraction from ruthenium has been revised (*552*) and distillation procedures necessary to "clean up" processed iodine from solids have been published (*256*).

Lederer (*305*) describes his carrier-free separation of cesium-131 by chromatography, while Mizzan (*354*) uses a phosphotungstate precipitation method to separate this element. Salutsky and Kirby (*471*) obtain carrier-free lanthanum-140 by precipitation of the barium parent as the nitrate. A new, simple, and rapid solvent-extraction method for separating cerium from fission products with methyl isobutyl ketone is reported by Glendenin and others (*161*), while Petrow (*423*) describes an ion exchange procedure for the determination of neodymium, praseodymium, and cerium in fission products. Stewart (*525*) suggests a rapid separation of tracer amounts of rare earth elements of the yttrium group using glycolic acid and cation exchange resins, while Nervik (*377*) recommends the separation of rare earth elements by a gradient elution method which tends to eliminate the large "tails" found on elution curves of the lower rare earths in standard methods.

Stevenson and Hicks (*523*) separate tantalum and niobium by extraction with diisopropyl ketone, while Van Cleve and McDonough (*558*) have developed an electroplating technique for thallium-204.

Natural Radioactivities and the Actinides. Hyde has recently made a compilation of radiochemical separation methods for the actinide elements, which he presented at the Geneva Conference (*229*). This excellent paper lists numerous references to separations for this group.

Lederer (*303*) studied the separation of lanthanum, actinium, and certain rare earth mixtures by continuous electrophoresis on paper sheets. Separations of radium from barium and its natural products are reported by ion exchange (*435*), paper chromatography (*478*), and precipitation (*179, 252*). Separation of actinium from its daughter francium-223 has been effected by paper chromatography (*420*), and an excellent general discussion of radiochemical methods for the isolation of francium (element 87) has been given by Hyde (*230*).

Articles describe the extraction of thorium, bismuth, and polonium from radium and lead by mesityl oxide (*332*) and the separation of uranium by filter paper partition chromatography (*443*). Three papers presented at the Geneva Conference give the viewpoints of the United States (*141*), the United Kingdom (*142*), and France (*164*) on the solvent extraction separation of uranium and plutonium from fission products, while a resume of recently declassified distribution coefficients for these elements in a number of solvents was presented in *Nucleonics* (*397*). The electrodeposition of plutonium is described by Moore and Smith (*357*).

TRACER APPLICATIONS

Only part of the many applications of tracers in analysis are outlined below. The extent of their use seems to be limited only by the ingenuity of the research worker. Small amounts of radioactivity are ideal for tracing the passage of material through chromatographic or electrophoretic apparatus, whether it be columns of ion exchange resins, alumina, or papers with one- or two-way flow. Many other types of analytical separations such as precipitations, extractions, and electrodepositions have also been studied with tracers.

Chromatography (Including Ion Exchange). The subject of ion exchange is being treated in detail in another review in this series, but much of the basic work in this field has been done using tracers. A number of references to the general application of ion exchange to analysis have been presented in two review papers (*290, 408*). Boyd and Soldano (*50–52, 511*) used tracers in investigating self-diffusion in different kinds of ion exchange resins. Tracers were important in the study of the use of ion exchange in the separation of traces of rare earth elements from uranium (*116*). The separation by ion exchange of phosphorus and iron (*592*), barium and strontium (*49*), antimony and tin (*266*), and scandium, lanthanum, and yttrium (*445*) has been studied. Using some 12 different tracers, Freiling and coworkers (*336, 589*) investigated the effects of different complexing agents on cation exchange separations. Bonner (*45*) discusses the ion exchange equilibrium involving rubidium, cesium, and thallium ions. Feldman and others (*134*) describe the elution of beryllium with citrate, while Radhakrishna (*446*) has studied separations of several natural radioactivities with Dowex 50. Osborn (*407*) has applied cation exchange resins to the analysis of insoluble materials and Lindner (*315*) has attempted to separate calcium-40 and calcium-45 by ion exchange. The Berkeley group (*159*) in their work on new elements in the transuranium region have speeded up the separation of the actinides to such a degree that separations can be made by cation exchange in a matter of a few minutes.

The anion exchange resins show great promise for effecting many rapid separations not previously possible. Kraus and his group at Oak Ridge have spent several years evaluating the separations possible with these resins, using tracers in most cases to determine the separations obtained. This work was summarized in the Geneva paper by Kraus and Nelson (*283*) and is documented in the previous papers by these authors (*281, 282, 284–287, 373–376*). Herber and Irvine (*205*) studied the bromide complexes of cobalt, copper, zinc, and gallium with these resins.

Lederer used tracers to study the paper chromatographic separation of different phosphate ions (*304*) and rhenium and technetium (*306*), while Dickey (*113*) reports similar separations of radium D, E, and F. Winteringham (*588*) has introduced a new method for the two-dimensional paper chromatography of radioactive substances. Fouarge (*144, 145*) used chromatographic columns of cellulose to separate barium and strontium. Gapon, Ivanenko, and Rachinskiĭ (*155*) used phosphorus-32 in a chromatographic study of phosphates, while Stokes and coworkers (*528*) used iodine-131 to study the chromatographic separation of *p*-iodobenzoates of certain sterols.

Strain and coworkers have used tracers in a number of their investigations of electrochromatographic separations (*129, 476, 477, 590*). Deimel (*107*) traced ribonucleic acids with phosphorus-32 in an electrophoretic separation, while Lederer (*307*) used sulfur-35 and phosphorus-32 to study the separation of acids in the same type of procedure.

Solvent Extraction. Tracers are of particular importance in solvent extraction studies. Hicks and Gilbert (*210*), Ellenburg, Leddicotte, and Moore (*127*), and Scadden and Ballou (*480*) describe solvent extraction of niobium; Nelidow and Diamond (*372*) investigated the solvent extraction behavior of molybdenum. Irving and Rossotti (*233*) used tracers in their research on the extraction of group IIIB metal halides, and Draganić (*119*) worked on the extraction of europium. Dyrssen and Dahlberg (*125*) extracted lanthanum, samarium, hafnium, thorium, and uranium with oxine and cupferron, while Peppard and others (*419*) developed procedures for the separation of gram quantities of ionium (thorium-230). Ishimori (*237*) studied the extractability of very small amounts of organometallic compounds.

Precipitation. In homogeneous precipitation studies, Gordon and coworkers have used tracers for determination of the amount of coprecipitation of thallium with silver chloride (*168*), strontium with barium sulfate (*169*), and manganese(II) on basic stannic sulfate (*170*). Salutsky, Stites, and Martin (*473*) have determined the fractionation of radium-barium mixtures in precipitation as chromates from homogeneous solution. Lima (*313*) presents calculations of the amount of a tracer carried with precipitates of its parent. Teicher (*543*) has used the tracer technique to study thoroughly the precipitation of barium carbonate. Bradacs and coworkers (*54*) experimented with various procedures for the determination of germanium with the help of radioactive germanium-71.

Behavior at Low Concentration. Schweitzer and coworkers made tracer studies of the behavior at low concentrations of lanthanum-140 (*486*) and sulfate and scandium ions (*485*). Granstrom and Kahn (*174*) used carrier-free cesium to investigate the adsorption of low concentrations of cesium on paper. Kahn and Wahl (*257*) studied the chemical behavior of iodine at low concentrations, while Stehney and Sugarman (*521*) describe experiments on the interchange of fission product bromine with carrier bromine.

Miscellaneous. Dean and Reynolds (*104*) developed a rapid method for the separation and determination of bismuth, antimony, and tin by controlled cathode electroanalysis using antimony and tin tracers. Ishibashi, Fujinaga, and Kusaka (*235*) used tracers to study the separation of copper, bismuth, and lead by a controlled potential method.

Milner and Smales (*349*) describe an absorptiometric determination of niobium in certain low grade minerals. They use niobium-95 to determine the yield of the separation procedure, so that it need not be quantitative. Boyd and Galan (*53*), in developing a satisfactory colorimetric method for niobium and tantalum in steels, used tracers to determine the completeness of precipitations at various steps in the procedure. Several other authors also report (*38, 279, 350*) use of tracers in developing procedures for tantalum or niobium in minerals or stainless steels.

Possible errors in the determination (*158*) of alkalies in silicates were studied by incorporating 1% rubidium-86 and 6% cesium-134 into soda glass. Huttig, Simm, and Glawitsch (*228*) examined the grinding process of glass using cobalt-60.

Harris, Stericker, and Spring (*195*) recommend a tracer method as most sensitive for indicating that a metal has been cleaned satisfactorily prior to electroplating.

Hine and coworkers (*214*) describe multiple tracer techniques using scintillation counting for chromium-51 and iron-59 or potassium-42 and sodium-24. Mai and Babb (*327*) also use scintillation counting for vapor phase analysis with sulfur-35.

A new, rapid tracer method for the determination of phosphorus in slags is reported (*409*) to give a precision within ±6.2%. Samarin (*474*) reviewed the use of tracers in the study of the production processes of steel and iron and Douglas (*117*) discussed the use of radioactive tracers to tag special steel melts. Voice, Bell, and Gledhill (*565*) report the use of radon to determine the gas flow in large ducts and chambers operating at high temperatures. Colding and Erwall report (*82*) on wear studies of irradiated carbide cutting tools.

Rushman (*468*) has written an excellent article reviewing the use of tracers in paint research. Vandone (*560*) has written on the same subject, while Calkins and others (*71*) used tritium to determine moisture gradients in attached protective coatings.

Hull (*225*) described the use of tracers in refinery control.

Van Bavel and Underwood (*557*) reviewed applications of tracers to measurements of the physical properties of soil in its natural state, while other workers (*25*) studied the determination of diffusible phosphate ions in soils by isotopic exchange. Lambert and others (*294*) compare three types of labeled soils used in detergency tests.

Auerbach and Gehman (*14*) suggest sulfur-35 for the study of sulfur solubility and diffusivity in rubber. The method is also applicable to carbon-14.

Spitzy (*517*) tested his procedure for the determination of blood iodine with radioactive iodine, while Toribara and Sherman (*547*) found beryllium-7 useful for their determination of beryllium. Spitzy and Skrube (*518*) were able to extend the sensitivity of an iodine detection method with iodine-131, while Kallee (*258*) used this same isotope to trace the separation of insulin by electrophoresis. Boothroyd and others (*47*) propose a procedure for determination of the carbon-14 distribution in labeled glucose. Brown and Jackson (*62*) offer a simple technique for the estimation of radioactive components of plasma containing iodine-131.

Lyon and Reynolds (*321*) studied the radioactive species present in reactor cooling water with a gamma-ray spectrometer.

INSTRUMENTATION AND MEASUREMENT

The field of instrumentation has progressed rapidly during the past 2 years. The Geiger counter, long the workhorse of radioactivity measurements, is being steadily replaced by the proportional and scintillation counter.

Taylor has reviewed recent developments in nuclear instrumentation (*541, 542*). *Nucleonics* continues to publish numerous articles on techniques for improving measurements, a number of which have been compiled into a book (*384*).

Considerable progress has been made in the detection of small amounts of radioactivity in carbon-14 and tritium dating work. These advances have been well recorded and documented to the original literature by the Geneva papers of Hayes, Anderson, and Arnold (*199*) on liquid scintillation counting of natural radiocarbon and of Johnston (*250*) on low level counting and the future of isotopic tracers. In a paper issued since the conference, Ballario and others (*20*) describe another apparatus for carbon-14 dating.

Kohman and Saito (*277*) have reviewed all these uses of carbon-14 and tritium dating and also have described other radiochemical contributions to the fields of geology and cosmology in their excellent review article. Curran (*97*) has also discussed methods for determination of geological age by radioactivity.

SCINTILLATION COUNTING

This method, one of the earliest used to detect radioactive disintegrations (*288, 365*), was generally discarded with the advent of the more versatile Geiger tube. About 6 years ago, however, with the availability of photomultiplier tubes capable of detecting and amplifying scintillations coming in rapid succession, the scintillation method again gained prominence. One of its primary advantages lies in the ability of the dense detecting medium (crystals of sodium iodide or stilbene) to detect gamma rays with high efficiency.

Morton (*362*) has presented an excellent review of recent developments in the scintillation counter field, as have Hayes, Anderson, and Langham (*200*) on the role of liquid scintillators in nuclear medicine. Other reviews of interest in this field have been written by Swank (*533*), Curran (*96*), and Birks (*40*), while *Nucleonics* has recorded (*386*) various advances reported at the 1954 Scintillation Counter Symposium in Washington, D. C.

Scintillation well counters and other modifications have been reported previously, but Loevinger and Feitelberg (*317*) have used this type of counter for assaying the beta-emitting phosphorus-32 with an efficiency for this isotope of 0.8 count per 100 disintegrations. Haigh (*184*) emphasizes the fact that the scintillation counter is ten times more sensitive for weak solutions of iodine-131 and iron-59 than a jacketed beta counter. Wagner and Guinn (*567*) recommend liquid scintillation counting for low specific activity work and Terentiuk (*544*) found a liquid scintillation counter was able to determine beta emission in solid samples. Furst, Kallmann, and Brown (*153*) report a method to increase the fluorescence in liquid scintillator solutions. Solon and coworkers (*512*) discuss the interpretation of background data from scintillation detectors and a breakdown of factors contributing to the background has been given (*398*).

Price and Hudson (*437*) report a method whereby both the specific activity and the position of a labeled material are determined simultaneously with a beta and ultraviolet photon counter. Hiebert and Watts (*211*) describe a fast coincidence circuit for measurement of tritium and carbon-14. Swank and Moenich (*534*) report a procedure for packaging photomultiplier tubes and Fischer (*139*) describes preparation of large-area plastic scintillators.

SPECTROMETRY

The scintillation techniques described above record many sizes of pulses coming from the crystal. Spectrometers, on the other hand, by recording only pulses of a given magnitude, take advantage of the fact that the pulse from the crystal is proportional to the energy of the particles initiating it.

Siegbahn (*500*) has edited an excellent reference book on nuclear spectrometry; another thorough review is given by Curran (*95*). Salmon (*470*) treats the analytical aspects of gamma spectroscopy. O'Kelley (*404, 405*), Hine (*213*), and Borkowski and Clark (*48*) have described types of scintillation spectrometers, while Miller (*347*) described a gamma proportional spectrometer. Problems and techniques of beta-ray spectrometry have been considered by Cook (*87, 88*) and Marshall (*333*).

The Hanford group gives procedures for analysis of radionuclide mixtures (*86*) using a gamma-beta scintillation spectrometer and describes a method of analysis for low energy gamma-ray emitters (*556*). Moteff (*363*) reports the energy spectrum of gamma rays from gross fission products, and Keneshea and Saul (*265*) present graphs showing contributions of each element to the beta activity in these same mixtures. Kahn and Lyon (*254*) present an excellent discussion on the use of spectrometers in radiochemical analysis and Kittel (*272*) describes the use of this type of instrument to determine uranium burnup. Rosenthal and Anger (*461*) recommend the use of a liquid scintillation spectrometer for counting tritium and carbon-14 labeled compounds.

When making measurements in which it is impossible to compare the sample against a standard, it is necessary to know the counting efficiency for gamma rays of different energy in the crystal. Kahn and Lyon (*254*) and McLaughlin and O'Kelley (*324*) give graphs of this information derived from calculations and substantiated with experimental data. Baskin, Demorest, and Sandhaus (*29*), Sterk and Wapstra (*522*), and Griffiths (*178*) give additional information of this type, while Rietjens and others (*455*) describe the influence of the distance between the source and the crystal on the counting efficiency. Heath and Schroeder (*203*) discuss the quantitative techniques of scintillation spectrometry as applied to the calibration of standard sources.

Gamma rays are slowed down in matter in several different ways. The gamma ray may give up its entire energy to an electron of the absorbing material. This monoenergetic photoelectron registers as a sharp peak on the scintillation spectrum. In a second possibility, known as the Compton effect, the gamma ray loses only part of its energy to the absorber and goes off in a different direction at a lower energy. If these secondary gamma rays escape from the crystal, the pulses from the original monoenergetic gamma rays will be recorded as a continuous smear of energies with a definite maximum energy. This causes difficulty in gamma spectroscopy, when Compton distributions from higher energy lines interfere with photopeaks of low energy lines. Bell (*32*) and Koch and Foote (*276*) recommend a large crystal to reduce the possibility of escape of the scattered gamma rays. Albert (*3*) and Peirson (*418*) utilize anticoincidence methods to minimize the Compton interference. Meyerhof and West (*346*) present their data on this escape peak.

In gamma spectrometry, as in other forms of spectrometry, it is possible to scan a spectrum of energies with a window of variable width. Numerous single-channel analyzers are available for this purpose. As it is preferable, however, to use a number of windows side by side to register pulses of particular energy groups simultaneously, multichannel analyzers of many types have been developed. Kelley (*264*) described these methods of pulse analysis in his Geneva paper, which includes an extensive bibliography. Engelkemeir and Magnusson (*130*) have also described a multichannel analyzer.

NUCLEAR EMULSIONS

While the nuclear emulsion is ideal for detecting individual radiations having peculiar properties, it is less satisfactory for routine measurements of integrated disintegrations. Thus although millions of films are used each year in film badge service and dental x-rays, the nuclear emulsion remains a curiosity to most workers assaying radioactivity.

Goldschmidt-Clermont (*165*) has presented an excellent review on photographic emulsions. Davenport and Stevens (*100, 101*) have used x-ray film to compare radioactivities. Coppens (*89*) applies nuclear plates to the measurement of radioactivity in liquids, while Mayr (*338*) uses these plates in activation analysis. Dudley (*121*) gives an excellent summary of the photographic detection of beta rays. Bhatnagar and Ghosh (*39*) suggest nuclear plates for the quantitative study of uranium in ore and point out that only by this method can the percentage of uranium present in the radioactive mineral portion of the ore be assayed. Katz and Chaikoff (*263*) separate compounds present in animal tissue on paper chromatograms, which are then identified by radioautographs. Laporte has devised a new method which he calls "kinetic nucleography" for the absolute determination of the activity of an alpha emitter using a nuclear emulsion (*151, 152, 295, 458*).

ABSOLUTE COUNTING

Most analytical determinations with radioisotopes are made by comparing the unknown with a standard of the same material to eliminate problems of geometry, scattering, absorption, mounting, etc. Where this is not possible, it is necessary to resort to absolute counting. One popular technique is to use the 4-pi counter, consisting of two hemispheres, in the center of which is counted a weightless sample on a very thin film. Houtermans, Meyer-Schützmeister, and Vincent (*218, 345*), Cohen (*80*), and Putman (*438*) have discussed the problems involved in these measurements. Pate and Yaffe (*414, 415*) have begun a series of articles dealing with the problems of absolute counting. Belcher (*31*) describes the use of liquid scintillation counters in absolute counting and Curtis and others (*99*) discuss absolute alpha counting.

STANDARDS

In any form of analysis it is important to have available suitable standards to check the measuring equipment used. Manov has written two excellent articles (*330, 331*) reviewing the situation regarding radioactivity standards. He includes numerous references to pertinent articles. Seliger and Schwebel (*490*) have discussed the standardization of beta-emitting nuclides, presenting comparison results from a number of laboratories for several NBS radioactive standards. Reynolds and Brooksbank (*453*) propose the use of thallium-204 as a standard for radioassay. Hall and coworkers (*163, 227*) describe methods for the precision counting of alpha particles with special reference to standard sources.

MEASUREMENT TECHNIQUES

There have been reported in the literature many special measurement techniques to adapt available counting equipment better to specific problems.

For measuring weak beta-emitting substances, there are described a flow counter sample changer with positive out-gassing (*209*), a proportional flow counter with high-humidity gas (*380*), a liquid sample beta counter (*118*), and a gas sample counting method for soft beta emitters (*381*). Problems encountered in the preparation of weak beta emitters for counting (*267, 463*) and self-absorption difficulties inherent in routine measurements of these emitters have been investigated (*98, 570*). Nader and coworkers (*369*) studied the performance of the internal counter operating both in the proportional and Geiger regions for several isotopes.

Low Level Counting. Davidon (*102*) described a nomogram for counting time, and Crouch (*93*) discussed the significance of very low counting rates. Sittkus, Ganz, and Remy (*501*) studied the background radiations, while Fafarman and Shamos (*131*) describe the effects of fall-out from an atomic blast on background counting rates. Miller and Leboeuf (*348*) describe the effect of high beta backgrounds on precision alpha counting. Wagner (*568*) describes methods for calculating the half life for low activities of short lived nuclides, and Riedel (*454*) discusses problems of statistical purity in nuclear counting. Veall and Baptista (*563*) describe a multitube gamma-counting apparatus for small samples.

Absorption Measurements. The energy of a radiation can be determined by absorption curves, and Harley and Hallden (*193*) describe a method for analyzing these curves graphically to identify the emitters. Phillips and Jenkins (*425*) report another approach to the same problem, while Ferro (*135*) has been successful in using gold absorbers to identify the activity. Because of the high atomic number of the absorber, the absorption is almost exponential and hence the gold absorption coefficient can be used as a characteristic property of a radioisotope. Baker and others (*18*) describe a very wide range absorption counting system which permits identification of particles from 10-k.e.v. betas to 5-m.e.v. alphas. Suttle and Libby (*532*) show that a wide variety of simple beta emitters give exponential absorption curves when the sample, absorber, and counter are placed close in cylindrical geometry. This forms the basis for a method of absolute measurement of beta radioactivity in solids, which is reliable to within about 5%.

Growth and Decay. Another property of radioisotopes is their half life. This property enters into calculations of radioisotope build-up and decay in irradiations and forms the basis of a nomograph drawn by Stehn and Clancy (*520*). Representation of the growth and decay of the natural radioactive families requires complicated sets of calculations. Articles relating to these calculations have been published by Kirby (*269, 270*), Kirby and Kremer (*271*), and Flanagan and Senftle (*140*).

Chromatography and Automation. With the advent of so much interest in paper and column chromatography, it is natural that there should be an effort to devise counting systems specifically for this technique. One group reports (*196*) using a 4-pi counter for scanning the paper chromatograms, while a second (*109*) uses a gas flow proportional counter and a third (*436*) uses a combination fluoroscope and Geiger counter. Several devices have been reported (*35, 73, 81, 309, 428, 587*) to scan paper chromatography strips automatically. Other automatic equipment has been designed for a scintillation well sample changer (*110*), a sample changer and recorder for dipping counters (*323*), an automatic fraction collector (*105*), and a windowless flow counter operating as a continuous counter (*128*).

CHEMICAL TECHNIQUES

So many notes in the literature fall into this category that it has been possible to point out only a few important sources of information. Papers presented at the Geneva conference by Fields and Youngquist (*136*) and Dismuke and others (*115*) describe hot laboratory facilities and techniques suitable for radiochemical problems. *Nucleonics* (*389*) devoted 65 pages to a special report on hot laboratories and Frederick (*148*) described a high-radiation-level analytical laboratory.

The emphasis placed on complicated equipment in much of the literature tends at times to give the erroneous impression that all radioisotope work requires quantities of specialized equipment. Bizzell (*41*) has listed certain basic equipment necessary for a radiochemical laboratory and suggests adaptations of simple, inexpensive equipment for this work. *Nucleonics* (*385*), in its annual buyers' guide, lists suppliers for all types of nucleonic equipment.

SAFETY

Underlying all applications of radioisotopes must be the principle of safety. The many articles written on the subject are summarized by Morgan, Snyder, and Ford (*358, 359*) in their recent reviews. Tabershaw and Harris (*537*) adequately describe the administrative problems of radiation protection and Barker (*26*) has edited a handbook that is used at Los Alamos for radiation monitoring. The National Bureau of Standards has issued two recent handbooks (*370, 371*) on radiation protection.

Three specific aspects of safety warrant particular mention here: the isotope-handling calculator of West (*578*) for calculating isotope hazard, and the surveys made by *Nucleonics* on commercial film badge service (*393*) and portable shielding material (*395*).

PATENTS

The revision of the Atomic Energy Act in 1954 has made a drastic change in the basic rights of individuals and companies regarding patents in atomic energy matters. Cole (*83*) has given a lucid discussion of these changes and of the regulations in effect at the present time.

SUMMARY

The application of radioisotopes to analysis has proved to be no cure-all for the problems of the analyst. In fact, in none of the articles described above has it been possible to make an analysis to within the accuracy or precision obtainable with the simplest of gravimetric procedures.

Fluharty sums it up well at the conclusion of his excellent chapter on the design of tracer experiments (*57*).

> Proper use of radioactive isotopes adds to the demand on other techniques rather than diminishes them. Only in a few cases will isotopes greatly simplify the problems encountered; rather they allow one to undertake problems which could not be tackled at all before. This is accomplished, however, by demanding the utmost, if not extensions of, old techniques, and their use may require many new concepts.

ACKNOWLEDGMENT

The preparation of this paper has been assisted by the United States Atomic Energy Commission and the Michigan Memorial Phoenix Project through their support of nuclear chemistry research at the University of Michigan. It is a pleasure to acknowledge the help of Rosemary Maddock and Marilynn Meinke for invaluable assistance in preparing and checking the manuscript, and of Edith Anders for her careful typing of the bibliography.

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**REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS**

Inorganic Microchemistry

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THE review of inorganic microchemistry is presented as an evaluation of trends in the methods, techniques, and tools of microanalysis. As part of the evaluation, selected topics are cited as being of particular significance, although the reviewer recognizes his inability to judge properly all of the individual advances in such a broad field. Critical examination and evaluation of details of procedure are especially difficult. Often, however, relatively restricted portions of an investigation may contribute key advances for general techniques. For this reason, a number of papers are cited which show specific aids and illustrate their uses. The reviewer considers methods of sampling, separating, detecting, and determining micro amounts of material regardless of the techniques employed.

Microchemistry embraces major portions of chromatography, ion exchange, extraction, light absorption spectrometry, emission spectroscopy, x-ray absorption spectroscopy, chemical microscopy, nucleonics and parts of various other electroanalytical methods. The separate reviews in these fields are anticipated, however, and only occasional mention of work involving such methods is made here.

The period covered by this review extends from the time of the 1954 review (*252*) through the articles abstracted in the October 1955 number of *Analytical Abstracts*. Of possible interest is the use of *Analytical Abstracts* as the general guide to the literature of analytical chemistry. This reviewer uses these abstracts (published by the Society for Analytical Chemistry and printed by W. Heffer and Sons, Ltd., Cambridge, England) because they can be obtained printed on one side and so can be cut out and pasted on punch cards. The abstracts are excellent and the cost is a modest 60 shillings. Because *Chemical Abstracts* can not be obtained printed on one side of the page only, the abstracts do not lend themselves to the direct transfer to punch cards.

A total of 1082 articles have been considered in preparing this review. Obviously, not all of these references can be cited and those that have been retained are not necessarily the only contributions of importance. The combined topics of all references have been classified for purposes of evaluating trends. Major review articles comprise 3% of the references, apparatus 3%, and special separation methods 47%. Of the separation methods, chromatography made up 40% of the list, masking (considered here as a separation tool, and consisting mainly of complexation) 23%, ion exchange 2%, and extraction 33%. There were a few references to volatilization methods of separation, and some uses were described for gathering agents. Precipitation remains as a standard means of separation and it is difficult to classify and count the number of such methods utilized. Many new and unique precipitation methods have been developed.

Of the 1082 inorganic microchemistry papers, 51% involved the use of organic reagents. Colorimetry was the most popular method of measurement. A total of 37% of the papers dealt with colorimetry, 12% with titrimetry, and 5% with gravimetry. Flame photometry has become increasingly important, with almost 5% of the articles selected devoted to this technique. Qualitative methods, mainly spot tests, constituted over 7% of the contributions reviewed.

BOOKS AND REVIEWS

A number of review articles have dealt with various aspects of inorganic microchemistry. Pollard (*190*) has discussed alumina, cellulose, paper chromatography, and ion exchange (in one of 17 excellent articles dealing with various phases of chromatography and appearing as a group). The principles and methods of chromatography have been discussed by Wells (*250, 251*) and by Dimler (*52*). Lacourt (*142*) has reviewed the methods of separating, detecting, and determining various metals. The uses of ion exchange techniques in analytical applications have been reviewed by Calmon (*35*), Pallaud (*178*), and Samuelson (*203*). An excellent article on methods of trace accumulation (*219*) reviews the applications of extraction, evaporation, electrolysis, ion exchange, precipitation, gathering, and chromatographic methods for isolating trace materials. An excellent bibliography is included.

Coulometry has been reviewed by Furman (*85*), and the discussions of high-frequency methods (*48, 109, 253*) are of interest. A discussion of microchemistry and ultramicrochemistry has been presented (*255*). The physical and chemical properties of dimethylglyoxime have been summarized in a comprehensive review (*28*), and an interesting discussion of the uses of metal complexes as redox indicators, masking agents, and other analytical aids has appeared (*95*).

A critical and authoritative appraisal of colorimetric methods for the determination of the platinum metals has been presented by Beamish and McBryde (*10*). A careful appraisal has been made of the procedures and the factors influencing the results of flame photometry (*185*); also, an excellent review of the sources of error in such methods has appeared (*191*), together with a survey of methods used in minimizing the various errors. Valuable reviews summarize methods available for determining individual metals. For example, Nutten has surveyed recent work on methods for determining potassium (*173, 174*), including procedures applicable for microanalysis. Geilmann and Gebauhr (*91*) have reviewed and critically discussed methods proposed for the detection of potassium, rubidium, and cesium. Reviews have appeared on the methods used in separating niobium, tantalum, and zirconium (*254*); analyzing titanium and titanium alloys (*45*); and separating and estimating beryllium (*206*).

Books of special interest for the microanalyst include one of flame photometry by Mavrodineanu and Boiteux (*157*), electrochemical methods by Delahay (*50*), and two general treatises on inorganic microanalysis by Duval (*56*) and Geilmann (*89*). The revision of the classic book on spot tests by Feigl warrants special attention (*63*).

INSTRUMENTS AND APPARATUS

A microbalance without a beam rider has been described by Pfundt (*182*). The balance is essentially a projection-type instrument. Quartz fiber balances have been described by Kuck, Altieri, and Towne (*137*) and by Richards (*197*). A vibrationless balance table has been designed by Gysel and Strebel (*107*). It consists essentially of a heavy plate suspended on springs which rest in a high viscosity damping material. Bush and coworkers have proposed an interesting micromanipulator having mechanical activation for coarse adjustments and thermal expansion units for fine control. The thermal control is obtained by electrical resistance heating (*34*).

Because of steadily increasing interest in solvent extraction methods, the extractors of Branica (*27*), Short (*214*), and Iffland (*119*) are of significance. An excellent microburet, developed by Gilmont (*99*), operates by means of a displacement plunger and is read by means of a dial micrometer. Volumes as small as 10^{-9} ml. can be read. A convenient microburet utilizing air flow displacement has been proposed (*239*), and Kirk has designed a useful self-adjusting delivery pipet (*105*). Gas absorbers have been described which are suitable for ultramicrogravimetric applications (*177*), and an absorber for mists and gases has been designed with the special needs of air pollution studies in mind (*200*). The apparatus described by Weisz (*245*) for generating gases and passing them into small volumes of liquids is of value because of its flexibility. Temperatures of the gases produced can be altered, and dilutions can be made by addition of indifferent gases without exposure to air. The British Standards Institution has given specifications for heating and cooling blocks (*30*) and for microbreakers (*29*). A double layer filter has been described (*127*) which permits an initial separation of coarse materials on a prefilter and a final separation of small crystals on a fine filter. It is claimed that microfiltrations can be speeded up through use of the device.

Modification and adaptation of the Beckman DU spectrophotometer to increase its value in microchemical work have been the subject of a number of papers. A Teflon insert has been proposed as a means of reducing the volume of solution required without shortening the light path (*212*). The insert diminishes the volume required to 1 ml. and yet permits uninhibited passage of the light from the monochromator. Two papers have described methods of adapting the Beckman spectrophotometer for use as a fluorimeter (*102*, *230*). The thermostated cell holder for the Beckman instrument is also of interest (*54*). An automatic servo-operated filter photometer is of value for applications on board ship or in mobile laboratories. Such an instrument has been designed by Snodgrass, Carritt, and Wooster (*218*). It operates on a double photocell design with automatic output balance from the cells. The signals from the photocells are balanced by a servo-motor adjustment made to the main slidewire. A potentiometric titration apparatus for use in ultramicroanalysis has been described by Alimarin and Petrikova (*2*). The techniques involved in conducting potentiometric titrations on 0.001-ml. samples are discussed, and the claim is made that errors do not exceed 2 or 3%.

Probably the most novel piece of equipment described recently is the so-called "ring oven" developed by Weisz (*246*). This is a relatively simple device which can be used to carry out complete schematic separations on a single drop of sample. The general design of the apparatus is shown in Figure 1. The discussion of its applications and the techniques involved in its use is deferred to the section on separations. Incidentally, the reviewer prefers the name "ring heater" to "ring oven." The word oven means a chamber of enclosed space and Weisz's apparatus is simply a heating ring surface.

SEPARATIONS

Without doubt, separations remain the analyst's best friend and biggest problem. Specific reagents or specific measurements of properties are very slowly becoming available, but at no time in the immediate future can we expect specificity without the use of conditioning reactions and separations. A tremendous amount of work is being done in developing separation methods, and many valuable techniques and procedures have been described.

Two clever and elegant microchemical techniques warrant special mention. The first is the "ring oven" (or better, ring heater) *technique* developed by Weisz (*246–249*). The method is based on the use of a heated ring (*Figure 1*) which serves as a means of fixing dissolved salts in a concentrated circle. The salts are transported through a supporting paper to the hot surface after having been added or extracted at a central spot, and the carrier solvent is evaporated by the ring heater, thus depositing the solute in a thin line and in an ideal state for subsequent spot test detection. By using the Weisz technique, it is possible to carry out a major scheme of separation using a single drop of original sample.

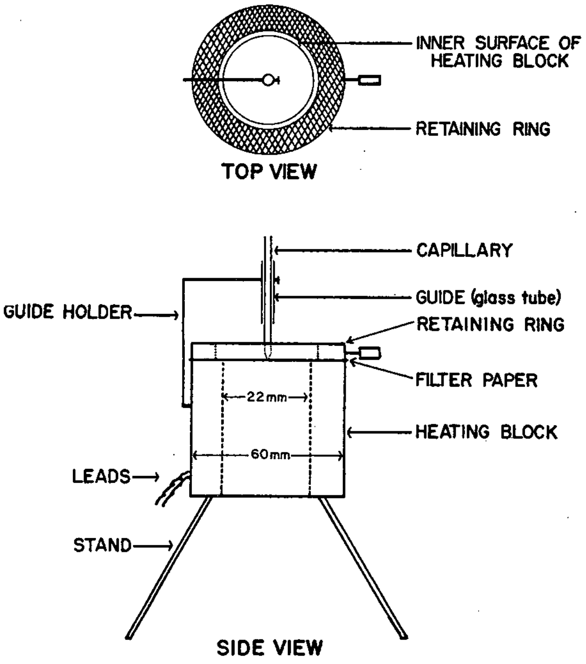

Figure 1. Ring oven

In a hypothetical example of a sequence of separations accomplished by means of the ring heater, an unknown is suspected to contain nickel, iron(III), lead, tin(IV), and copper(II). A single drop of the neutral sample is placed in the center of a piece of filter paper (50 mm.) and exposed to hydrogen sulfide using the gas applicator (*246*). The filter paper is then placed on the ring heater with the spot of precipitated sulfides centered over the annular space. A drop of 0.1*M* hydrochloric acid is added to the center of the spot by means of a capillary. The nickel and iron precipitates are dissolved and transported to the heated ring where the solvent is evaporated. After the residual sulfide precipitate has been washed twice, it can be assumed that a quantitative separation has been achieved. By means of a cork borer, the sulfide spot is stamped out. The outer ring is divided and one sector tested for iron—e.g., α,α-dipyridyl—and the other tested for nickel—e.g., dimethylglyoxime. The disk containing the sulfides is then exposed to bromine fumes, placed on a fresh circle of filter paper, and centered on the heater. A drop of ammonia is added to the spot, which is then washed with dilute ammonia to transport the dissolved copper to the ring, where it is deposited through evaporation. Any tin will remain fixed in the center spot which can be cut out for subsequent testing. The copper (ring) can be detected by means of the dithiooxamide test, and the tin can be dissolved and tested by means of cacotheline. The whole scheme of separation can be carried out in 15 or 20 minutes.

The second separation procedure given special mention is the ion exchange procedure described by Fujimoto (*83*, *84*). This is a microchemical modification of a standard technique, but it is so simple and rapid that it should prove a valuable tool. The method can be illustrated by reference to a cobalt test: A drop of the neutral test solution is placed on a white spot plate together with a few grains of a colorless or pale-colored resin—e.g., Amberlite IRA 400. A drop of ammonium thiocyanate is then added and a blue coloration begins to appear around the edges, or throughout the grains of resin. Kakihana and Murase (*128*) have used Amberlite IRA 411 grains as collectors for germanium.

A more conventional use of ion exchange materials for special microchemical applications has been suggested by Kul'berg and Lenskaya (*139*). They use a small column of exchange resin to concentrate samples, shift equilibria, or determine the charge on complexes. They suggest a test for carbonate in which a grain of cationite is added to a drop of sample together with a drop of phenolphthalein. A positive test is indicated by the formation of bubbles and decolorization. Similar spot tests are suggested, based on the use of individual particles of resin which have been used to concentrate the ions under examination. No elution is required, and good sensitivity can be obtained for the selected tests. The value of conventional ion exchange methods in solving certain problems of special difficulty can be illustrated by the work of Rieman and his associates who have developed a method of separating various phosphates (*150*). Mixtures of ortho-, pyro-, tri-, trimeta-, and tetrametaphosphate can be resolved through use of an exchange column together with two or three eluting solutions.

As a means of comparison, reference is made here to chromatographic separations. Mixtures of orthophosphates and polyphosphates have been handled through use of paper partition chromatography (*93*). Various acidic and alkaline developers are used, and ammonia is added to the separated phosphates to secure final elution. After elution, colorimetric measurements serve to give good quantitative values on relative concentrations of the separated phosphates. Ebel has described the use of one- and/or two-dimensional paper chromatography in the detection and estimation of the various oxy- acids of phosphorus (*58*). Crowther (*47*) has proposed a simple, general method of filter paper chromatography for the analysis of the various phosphates. Lederer has used paper chromatography to separate certain cations from phosphate (*149*).

Other separations made with chromatographic techniques can be illustrated by a few selected papers. Ladenbauer proposed (*143*) the semiquantitative determination of germanium with separation from the important possible interfering metals by paper chromatography. Berg and McIntyre accomplished complete separation of various mixtures of cobalt(II), iron(III), nickel, and manganese(II) through paper chromatography of the 2-thenoyltrifluoroacetonates (*15*).

The use of impregnated papers for chromatographic separations is of special interest for the microanalyst. Fernando has used paper impregnated with 4-hydroxybenzothiazole for the simultaneous separation and detection of a number of metals (*68*). Fernando and de Silva (*69*) have also worked along this line. A unique approach to separation and detection is that of Milone and Cetini, who have employed diffusion through gels to obtain separation into bands of precipitates (*164*). The position and nature of the separate bands serve for identification of the diffused salts.

Extraction methods are rapidly being established as a very valuable microanalytical tool. This old technique is now becoming widely appreciated, and many important applications are appearing in the literature. Typical examples of the application of solvent extractions are given to show some of the scope of the technique. Many specific applications of valuable separations could also be cited. Wadelin and Mellon (*243*) have separated molybdophosphoric and other heteropoly acids by extraction into various solvents. The separations can be combined with spectrophotometric determination. DeSesa and Rogers have suggested a very neat means of analyzing mixtures of molybdosilicic, -arsenic, and -phosphoric acids (*51*). The combined heteropoly acids are measured spectrophotometrically. The phosphorus is then estimated after extraction with isopentyl acetate and the arsenic is subsequently extracted from ethanolic solution and determined. The elimination of phosphate interference with certain ethylenediaminetetraacetic acid titrations has been made using solvent extraction (*43*).

The future of solvent extraction in the separation of inorganic materials can be implied from an article by Pohl (*187*). Pohl suggests that trace metals can be determined by first removing major amounts of iron, aluminum, and titanium by a combined chloride and thiocyanate extraction. The trace metals are then isolated and concentrated by extraction into chloroform, using tetramethylenedithiocarbamate and dithizone as extracting addenda. Very efficient extractions of a number of metals can be made using thiocyanate. Tributyl phosphate is an excellent solvent for such extractions (*158–160*). Ether plus tetrahydrofuran is especially recommended as a solvent for extracting iron and aluminum thiocyanates (*220*). Probably the most generally useful extracting agents are, in addition to thiocyanates, diethyldithiocarbamates (*25, 166, 186, 221*), dithizone (*81*), and oxine (*228*). Acetylacetone is an excellent extracting agent, serving as both the complexing addendum and solvent (*222*). It reacts with over 60 metals.

The various approaches to avoiding the use of hydrogen sulfide are of interest. The general value of sulfide separations is unquestioned, but there are many good reasons to develop modified or substitute approaches to such precipitations. The various sulfide-producing reagents that give homogeneous precipitations are important (*232*) because they often give cleaner precipitates that are easier to handle. Sulfhydryl groups in organic reagents are also advantageous because they can serve as group precipitants, giving sharp separations. Thionalide is the best known example of this latter type of reagent (*188*). The use of thallium as a carrier with homogeneous sulfide precipitation for various metals has been proposed by Pohl (*187*). Mention of coprecipitation recalls the studies of Kuznetsov on the use of organic coprecipitants (*140, 141*). As a matter of clarification, it should be pointed out that the work refers to the use of coprecipitants, but does not entail coprecipitation in the usual sense encountered in gathering operations. Kuznetsov really uses simultaneous precipitations in order to obtain separation efficiencies. In one sense, he employs an extraction procedure with solid solvents.

Increasing interest is evidenced in the applications of amalgamation and electrolysis at mercury cathodes. For example, zinc and cadmium have been treated with metallic mercury to

Table I. Common Masking Agents[a]

Metal	Agent	Metal	Agent
Ag	CN^-, NH_3, $S_2O_3^{--}$, Br^-	Pt metals	
Al	F^-, $C_2O_4^{--}$, AcO^-, cit.	Os	CN^-, thiourea
As	S_2^{--}	Pd	$S_2O_3^{--}$, I^-, CN^-, NH_3, NO_2^-, CNS^-
Au	CN^-, Br^-, $S_2O_3^{--}$	Pt	I^-, NH_3, CN^-, $S_2O_3^{--}$ NO_2^-, CNS^-
B	F^-, hydroxy acids	Ir	Thiourea, cit., CNS^-
Ba	EDTA	Rh	Thiourea, cit.
Be	F^-, EDTA, tart.	Ru	Thiourea
Bi	EDTA, tart., cit., I^-	Sulfur family	
Ca	EDTA, polyphosphates	S	CN^-, S^{--}, SO_3^{--}
Cd	CN^-, cit., EDTA, I^-, NCS^-, $S_2O_3^{--}$	S^{--}	S
Ce	F^-	SO_3^{--}	Hg^{++}, HCHO
Co	CNS^-, CN^-, $S_2O_3^{--}$, cit., EDTA, NO_2^-	Se	S^{--}, SO_3^{--}
Cr	AcO^-, EDTA	Te	I^-
Cu	CN^-, tart., cit., $S_2O_3^{--}$, EDTA	Sb	Tart., cit., I^-, S_2^{--}
F	HBO_3	Sn	$C_2O_4^{--}$, F^-, I^-, tart., cit.
Fe	Tart., cit., $C_2O_4^{--}$, F^-, PO_4^{---}, $P_2O_7^{----}$, mal., $S_2O_3^{--}$, EDTA	Sr	EDTA
Ge	$C_2O_4^{--}$, F^-	Ta	F^-
Hg	I^-, SO_3^{--}, CN^-	Th	F^-
Mg	EDTA	Ti	F^-, $H_2O_2 + SO_4^{--}$
Mn	F^-, $C_2O_4^{--}$, $P_2O_7^{----}$, tart.	Tl	Cl^-, CN^-
Mo	NCS, $C_2O_4^{--}$, H_2O_2, cit.	U	F^-, $C_2O_4^{--}$, tart., cit., CO_3^{--}
Ni	Tart., cit., mal., EDTA, CN^-, NCS^-	V	CN^-
Pb	AcO^-, $S_2O_3^{--}$, cit., EDTA	Zn	CN^-, NCS^-, cit., EDTA, tart., glycerol
		Zr	F^-, SO_4^{--}, tart.
		CN^-	HCHO

[a] Tart. = tartrate; cit. = citrate; mal. = malonate; EDTA = ethylenediamine tetraacetate.

form an amalgam. By partial anodic dissolution, all of the zinc can be dissolved, leaving the bulk of the cadmium. This method (*208*) can be used in setting up polarographic analyses of small amounts of zinc in the presence of large quantities of cadmium. Gardiner and Rogers (*88*) have developed a submicrogram method for coulometrically determining cadmium and zinc, which employs the known principle of deposition on mercury. The separation of traces of manganese on continuously renewed mercury cathode has been advocated (*46*), and interfering metals can be removed over the mercury cathode in the determination of vanadium (*213*).

Little has been said concerning masking. While this is not a separation method in the physical sense, it does serve the same purpose and so is mentioned in this light. So far as the author knows, there is no general source of information regarding masking agents likely to be of value in sequestering any given ion. It seems appropriate to begin recording masking agents for general reference, and Table I is provided as a start along such lines. The table is not complete, nor is the information new, but casual investigation indicates that the average chemist might expect some guidance from it in routine problems.

A summary of separation methods is given in Table II. Although this table includes masking agents, special mention is made of the paper of Kato and Takei (*130*) who used a soluble form of the familiar reagent diethyldithiocarbamate as a masking agent. The compound used, 2-hydroxyethylammonium 2-hydroxyethyldithiocarbamate, serves to sequester many metals and no phase separations are involved.

ORGANIC REAGENTS

The progress of inorganic microchemistry is closely allied with the development of organic reagents. New reagents are steadily appearing. There is also steady progress in the knowledge of the chemistry and applications of the established reagents. A discussion of the factors to be considered in choosing organic reagents has been presented by Williams (*260*). Yatsimirsky has discussed the "weighting effect" of substitution on reagent molecules (*262*). He points out that the substitution for a hydrogen atom in a —CH group leads to predictable changes in solubility according to the nature of the substituted group. This is an extension of Feigl's earlier classical work. Kul'berg and Davydova (*138*) have discussed the effect of substituting —$N(CH_3)_2$ and —$N(C_2H_5)_2$ groups for hydrogen on the benzylidene group of benzylidenerhodanine. Turkevich has studied the thioamide group, —C(SH):N—, and proposes it as an iron and bismuth "specific" grouping (*237*). Smith and his coworkers have done classical work on the iron-specific group, —N=C—C=N—, and they continue to contribute along these lines with systematic investigations (*257*). Similar work of great significance is being done by Hoste, Gillis, and their associates.

With the introduction by Hoste in 1950 of 2,2′-biquinoline (cuproine), a truly valuable reagent became available. Cuproine and similar compounds can be considered as the ideal reagent in regard to specificity, and many applications are being made in the determination of copper. Some general applications of cuproine have been summarized by Hoste, Eeckhout, and Gillis (*116*). Gillis, Hoste, and van Moffaert (*97*) have presented a means of synthesizing the dimethyl derivative of cuproine, 4,4-dimethyl-2,2′-biquinoline. This compound forms penetration complexes with copper(I) which have 12.7% greater absorptivities than the parent compound (*97*). Neocuproine, 2,9-dimethyl-1,10-phenanthroline, has been studied by Gahler (*87*) in an extension of the original work by Smith and McCurdy. Other studies of cuproine serve to confirm the usefulness and reliability of this reagent (*94*, *181*). The fundamental studies of Freiser and his group are of importance in the development of organic reagents. Four new contributions in the series have appeared recently (*38*, *39*, *124*, *125*).

Miscellaneous studies of importance have appeared recently. Of special value is the work by Belcher and his group on the investigation of substituted benzidines (*12*). The sulfate of 4-chloro-4-aminodiphenyl is very insoluble and will undoubtedly prove a valuable substitute for benzidine. Free chlorine can be determined nicely with 3,3-dimethylnaphthidine (*11*). Belcher and coworkers have investigated the analytical properties of 8-hydroxy-5-, -6-, and -7-trifluoromethylquinolines (*14*) and mandelic acid together with its halogen-substituted derivatives (*13*). Oesper and Fulmer have prepared and studied two fluorenyl derivatives of cupferron, which seem promising as a reagent for

Table II. Methods of Separation

Subject	Operation	Reference
Mo	Extn. into ether (HCl soln.)	(*3*)
Halogens	Abs. on Cu powder (from air)	(*202*)
Ga	Extn. into ether (as acetylacetonate from HCl)	(*261*)
	Anion exchange as oxalate complex	(*19*)
	Extn. into ketones or ether (HCl soln.)	(*163*, *176*)
	Extn. into CCl_4 (HCl soln.)	(*210*)
	Ion exchange (HCl elution)	(*134*)
	Extn. into acetylacetone	(*223*)
Ge	Pptn. as GeS_2 (thioacetamide)	(*9*)
	Distn. as $GeCl_4$	(*9*)
	Paper chromatography	(*144*, *145*, *148*)
	Ion exchange	(*134*)
	Copptn. with Cu (as the sulfide)	(*16*)
	Extn. into CCl_4 (HCl soln.)	(*70*)
	Extn. into ether (HBr soln.)	(*146*)
In	Ion exchange (HCl elution)	(*134*)
	Extn. into acetylacetone	(*223*)
Al	Extn. into acetylacetone	(*223*)
	Extn. into ether plus tetrahydrofuran (as thiocyanate)	(*220*)
Fe	Extn. into ether plus tetrahydrofuran (as thiocyanate)	(*220*)
Ta and Nb	Extn. into diisopropyl ketone (containing HF)	(*224*)
	Chromatography on cellulose	(*118*, *161*)
	Extn. into ether (as thiocyanate)	(*244*)
	Extn. into diisopropyl ketone (from HCl)	(*113*)
	Pptn. (Nb) with tannic acid + cinchonine	(*162*)
	Extn. into $CHCl_3$ or CH_2Cl_2 (with tribenzylamine)	(*59*)
	Extn. (Nb) into *n*-butyl ether (with butyl phosphate)	(*209*)
Th	Extn. into mesityl oxide	(*82*)
Ce	Extn. of cupferrate into *n*-butyl acetate	(*108*)
Zn	Extn. into *n*-butyl ether (with butyl phosphate)	(*209*)
Sc	Chromatography on paper	(*122*)
Be	Extn. into $CHCl_3$ (as the butyrate)	(*7*, *229*)
Alkali metals	Chromatography (paper)	(*33*)
Platinum metals	Distn. (Os and Ru)	(*256*)
	Extn. (Os) into $CHCl_3$ or CCl_4	(*207*)
Phosphates	Extn. into 1-butanol + $CHCl_3$	(*43*, *243*)
	Extn. into iso-pentyl acetate	(*51*)
Pb	Mask with 2,3-dimercaptopropanol	(*193*)
	Copptn. with Tl_2S	(*188*)
Bi	Mask with 2,3-dimercaptopropanol	(*193*)
Cr	Copptn. with Tl_2S	(*188*)
Zn	Extn. with methyldioctylamine in trioctylamine	(*153*)
	Subln. at 1100° C. in current of H_2	(*92*)
	Copptn. with Tl_2S	(*188*)
U	Extn. with CCl_4 containing dibutyl hydrogen phosphate	(*225*)
Mn	Copptn. with Tl_2S	(*188*)
Cu	Extn. with DEDTC into CCl_4	(*41*)
	Extn. with 1,10-phenanthroline into 1-octanol	(*258*)
	Mask with thioglycolic acid	(*180*)
	Mask with thiourea	(*132*)
	Copptn. with Tl_2S	(*188*)
Co	Extn. with DEDTC into CCl_4	(*41*)
	Copptn. with Tl_2S	(*188*)
Ni	Extn. with DEDTC into CCl_4	(*41*)
	Copptn. with Tl_2S	(*188*)
Sn	Mask with thioglycolic acid	(*180*)
	Distn. with HCl and HBr	(*62*)
	Extn. into benzene (in presence of iodide)	(*67*)
	Extn. into Hg	(*215*)
As	Extn. into CCl_4 (HCl soln.)	(*70*)
Ag	Extn. into 1-butanol with dithio-β-isoindigo	(*86*)
Te	Extn. with DEDTC	(*26*)

extracting and determining iron (*175*). Carmine red has been proposed as a color reagent for thorium (*61*), 2,5-dihydroxy-1,4-benzoquinone serves as a scandium reagent (*189*), and tin can be identified by means of 2-aminoisophenoxaz-3-one and 2-acetamidoisophenoxaz-3-one (*201*). A new reagent for zinc, 2-carboxy-2-hydroxy-5-sulfoformazylbenzene, has been described (*199*), and 3,3-diaminobenzidine has been proposed (*117*) as a sensitive reagent for selenium well suited for spectrophotometric measurement (at 348 mμ). The studies of Hoffman and coworkers on qualitative organic reagents for osmium are of interest (*114*), as are the investigations of reagents for antimony (*17*). Useful investigations of substituted dithiocarbamates have been made (*154*), and studies of various thiosemicarbazones (*106*) have disclosed potential reagents for copper, silver, cobalt, and mercury. Duval and Wadier have suggested the use of 5,5-dimethylcyclohexane-1,3-dione as a means of detecting mercury (*57*). Cyclohexanedionedioxime (nioxime) has been investigated from the standpoint of iron interferences in the determination of nickel (*8*), and cycloheptanedionedioxime has been subjected to thorough study (*96*) as a nickel reagent (spectrophotometric). Gillis and colleagues (*98*) have suggested isonitrosodimedone for use in detecting cobalt microscopically.

Boron has been the subject of considerable study. The following reagents have been investigated: various dihydroxyanthraquinones (*42*), curcumin in acetone solution (*216*), carmine (*49*), 1,1-dianthrimide (*101*), and tetrabromochrysazin (*263*). Mention of boron leads to summarizing remarks relating to the use of tetraphenylboron as a potassium reagent. Development of this reagent really began in 1951, but the steady output of work dealing with it since confirms the previous predictions of value. Some of the current work with tetraphenylboron includes an amperometric method for potassium (*131*), a turbidimetric method (*198*), and various titrimetric procedures (*76*, *78*). The gravimetric method is well established (*90*).

In summarizing the section on organic reagents it seems fitting that reference be made to the well known reagent ethylenediaminetetraacetic acid (EDTA) which is an ideal example of the versatility of many of the organic reagents. EDTA can well serve as a reminder that it is not always necessary to seek specific reagents.

The following references are cited to show the broad value of ethylenediaminetetraacetic acid: A conventional microtitration of iron has been devised (*72*) employing a visual (redox) indicator; EDTA has been employed as a masking agent for removing various cation interferences in the colorimetric determination of uranium with dibenzoylmethane (*192*); ultraviolet spectrophotometric estimation of iron is possible using EDTA as the absorbing addendum (*240*); various EDTA titrations of metals can be made using spectrophotometric detection of end points (*231*), including simultaneous titrations (*238*); amperometric titrations have been demonstrated (*147*); and EDTA has been used with potentiometric or high-frequency (*110*) detection of titrimetric end points. It may also serve as a primary standard (*18*).

GRAVIMETRY

Ma, Kaimowitz, and Benedetti-Pichler (*152*) have recommended inorganic fiber filter paper for use in microanalysis. Among the methods and reagents that have appeared recently that pertain to microgravimetric analysis are trimethylphenylammonium iodide (*32*), used for determining bismuth; thiourea dioxide (*172*), a reagent for tin; and the estimation of germanium using 12-tungstogermanate (*183*).

TITRIMETRY

A coloriscopic capillary for the titration of 0.5-cu. mm. volumes of samples has been described. The capillary is drawn from thermometer tubing and color changes of indicators are observed microscopically (*55*). A number of novel and useful titrimetric methods have been reviewed by Elving (*60*). The mercurimetric chloride procedure is not new, but is finding wider usage (*236*). There is also a continued interest in electrometric titrations on a microchemical scale. Potentiometric titrations can be made on the microgram level (*2*, *4*), and amperometric (*135*) and coulometric (*151*) titrations are proving well suited for micro work. Photometric titrations will undoubtedly find further use in microanalytical laboratories (*100*).

Table III. Ultraviolet Inorganic Spectrophotometry

Metal	Absorbing System	Wave length, mμ	Reference
Sb	Sb + HBr	366	(*168*)
	Sb + thiourea	320	(*171*)
Sn	Sn + HBr	200–330	(*170*)
Bi	Bi + HBr	370	(*226*, *167*)
Fe	Fe + HBr	280–310, 365–490	(*169*)
Cr	Cr + EDTA	380	(*103*)
Mo	Mo + thioglycolic acid	365	(*259*)
	Mo + NCS^-	320, 460	(*155*)
V	Orthovanadate	270	(*104*)
Pt	$PtCl_6^{--}$	262	(*133*)
Pd	$PdCl_4^{--}$	370	(*40*)
Ce	Ce + H_2O_2	320	(*233*)
Nb	Nb + HCl	281	(*129*)
	Nb + NCS^-	386	(*112*, *162*, *244*)
Ta	Ta + pyrogallol	325	(*53*)
S	SO_4^{--} + benzidine	250	(*5*)
Te	Te sols	240–290	(*123*)

Many microtitrations are now being made based on the use of EDTA. These include titrations of calcium and magnesium (*6*, *37*, *75*), uranium (*111*), aluminum (*80*, *234*), zinc (*227*), manganese (*77*, *79*), palladium (*71*), thorium (*80*), indium (*77*), gallium (*74*), and rare earths (*73*).

Attention is directed to the work of Bobtelsky and his coworkers (*20–24*) on the use of heterometric titrations. The method is based on conventional precipitation reactions which are followed photometrically (red filter) to determine the titration end points. Various masking agents, used with appropriate pH control, are employed to establish selectivity. There is some merit in the method.

COLORIMETRY

Over a third of the articles considered as dealing with inorganic microanalysis involved colorimetric measurements. The excellent review of light absorption spectrometry makes further review of this general subject superfluous here. There has been a great interest in ultraviolet spectrophotometry during the past two years, however, and Table III is included to show some of the scope of this important extension of inorganic spectrophotometry. Buck, Singhadeja, and Rogers (*31*) discuss some inorganic ions in aqueous solutions.

SPOT TESTS AND OTHER QUALITATIVE TECHNIQUES

A significant proportion of the new spot tests are based on fluorescence phenomena. Because fluorescence reactions are reviewed elsewhere, only brief mention of some of the more significant tests is made here. Thallium can be detected through the fluorescence of the rhodamine B complex (*66*). Benzoin (*195*) and resacetophenone (*194*) serve as fluorescent reagents for germanium, 2-salicylideneaminophenol gives a fluorescence with gallium (*179*), tin can be detected through its reaction with morin (*64*), and 2-*o*-hydroxyphenylbenzothiazole forms a salt with beryllium that gives a strong blue fluorescence (*115*). A number of new spot tests have been proposed that occur in nonaqueous media. Carlton (*36*) has proposed a test for bismuth based on the reaction of dithizone in molten naphthalene. The test is sensitive to 0.004 γ of bismuth. Seely has employed a gelatin-glycerol medium for detecting minute quantities (10^{-10} to 10^{-15} gram) of particles, particularly air-borne dusts. Appropriate reagents are used and the test results are observed microscopically (*211*). A test for chloride (*184*) is based on the use of gelatin impregnated with mercury(I) fluorosilicate. The test is especially useful in counting chloride particles in the air.

A test for palladium has been proposed using piaselenole (*264*). A test for cadmium in the presence of copper, lead, and tin can be made using a capillary separation to eliminate interferences (*242*), and cacotheline has been suggested as a reagent for iron (*196*). Chromazurol S serves as a very delicate reagent for beryllium (*235*). One part of beryllium in 4,500,000 can be detected. Sodium rhodizonate is recommended by Feigl (*65*) as a reagent for calcium. Some practical examples of the test are given showing its use in the examination of minerals.

Microscopical (crystal morphology) tests have been proposed (*241*) for indium, thallium, and antimony (using quinoline); indium, antimony, tin, and thallium (using 8-quinolinol); and for vanadium, tungsten, molybdenum, titanium, thallium, antimony, tin, and indium (using dibromo-8-quinolinol). Microscopical tests for cyanide have been developed (*136*), and the value of crystal properties in identification has been discussed (*126*).

MISCELLANEOUS

Radiochemical methods are finding numerous applications in microchemical work in spite of obvious physical restrictions. Activation analysis is of special value, having excellent sensitivity and broad application. Recent papers in this field include the determination of trace impurities in silicon (*121, 165*) and in iron (*1*). Rubidium and cesium in sea water have been estimated by radioactivation (*217*). Micro amounts of potassium can be determined by precipitating the cobaltinitrite, containing cobalt-60, and measuring the activity with a Geiger counter (*120, 204, 205*).

Marple and Rogers (*156*) have used mercury-plated platinum electrodes in a rapidly stirred solution for polarographic and coulometric determinations. Stirring yields a sixfold increase in the polarographic step height. As little as $10^{-8}M$ lead ion can be determined by the coulometric measurement.

The great interest in flame photometry must be noted. Without question, this approach to the determination of alkali, alkaline earth, and many other metals must be considered as a standard microanalytical procedure.

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REVIEW OF FUNDAMENTAL DEVELOPMENTS IN ANALYSIS

Organic Microchemistry

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IN THIS biannual review the author has selected those papers which to him seemed to indicate current interest and trends in the field of organic microchemistry, and has not tried to include all the publications on the subject. Another reviewer by stressing different aspects of the subject undoubtedly would have cited a number of papers not included in this review. As it was impossible to test in the laboratory even a small fraction of the methods described, selection of the publications to be stressed was a most difficult task. This was particularly true of those papers which described modifications of existing methods, because frequently only a small change in procedure or apparatus results in a greatly improved method.

CARBON AND HYDROGEN

Much of the work reported during the past 2 years has dealt with the two primary sources of trouble in the carbon and hydrogen determination—namely, the removal of the oxides of nitrogen and the elimination of errors that occur when some of the less common elements (in organic compounds) are present. This indicates that the methods in use today generally produce satisfactory results for the usual organic materials. It does not mean that there is no room for improvements nor that better methods will not be devised, but that the more pressing problems are the two mentioned above.

Numerous absorbents for nitrogen oxides have been proposed to date and a critical study of their efficiency and capacity seems to be desirable. Of equal importance would be a readily acceptable method for testing those qualities of an absorbent, so that not only the known absorbents could be compared but new materials could be evaluated.

Cropper (*13*) reported the results of a study of the efficiency of different preparations of lead dioxide. The efficiency was found to depend on the particle size of the lead dioxide; large particles had a low capacity and small particles a high capacity but led to erratic values. Consequently, a medium-capacity material was required for uniform performance. Fortunately, this type of material can be made by recommended methods. A new absorbent for the oxides of nitrogen was proposed by Cross and Wright (*14*). This was trishydroxylamine phosphate which, when used in an absorption tube between the water and carbon dioxide absorbers, removed the nitrogen oxides efficiently, increased the accuracy of the carbon values for nitrogen-containing compounds, and improved the precision of the results for nitrogen-free materials. Hussey, Sorensen, and DeFord (*23*) also claimed increase in precision with ammonium sulfamate dispersed on silica gel in an absorption tube instead of lead dioxide in the combustion tube. Abramson and Brochet (*1*) recommended diphenylamine and sulfuric acid on alumina as absorbent for the oxides of nitrogen.

The determination of carbon and hydrogen in organosilicon compounds was studied by Klimova, Korshun, and Bereznitskaya (*25*), and the method they proposed not only eliminated the errors caused by the formation of silicon hydride and prevented carbide formation, but also permitted the simultaneous determination of the silicon. To do this, chromic oxide on asbestos was placed in a special sample holder and volatile silicon compounds were made to react with the chromic oxide to form silica, which was retained in the tube and weighed. Klimova and coworkers (*26*) used the same technique when analyzing organophosphorus compounds, with equally good results. Even at 900°C. the phosphorus pentoxide was retained on the asbestos and the phosphorus could usually be determined with fairly good accuracy.

Another element which causes erroneous values if present in very large amounts is fluorine. Bodenheimer and Goldstein (*8*) developed an equation for correcting the hydrogen values obtained for fluorine compounds. They found that the hydrogen values should be diminished by $^1/_{57}$ of the percentage of fluorine in the compound. A practical approach to the problem of fluorine in the carbon and hydrogen determination was the interesting method for the simultaneous determination of carbon and fluorine described by Freier, Nippoldt, Olson, and Weiblen (*16*). Combustion was by moist oxygen in a quartz tube, the hydrofluoric acid formed was determined by acid-base titration, and the carbon dioxide was absorbed by Ascarite. Good results were obtained for a number of different compounds.

Kuck, Altieri, and Towne (*28*), using the Garner balance, made a critical study of diffusion rates through the capillaries of absorption tubes. Diffusion rates were related to capillary size, and, as expected, the larger the capillary the faster the rate. Standard tubes with 0.25-mm. capillaries filled with oxygen lost weight for about 2 hours, then gained at the rate of 0.201 γ per minute. The standard error of estimate for the Ascarite tube was ±1.65 γ and for the Drierite tube ±2.65 γ. Mitsui (*36*) has been actively designing and modifying microchemical apparatus, including many of the more modern and desirable features, and also critically examining such points as design of absorption tubes and preparation of reagents. For determining very small amounts of carbon dioxide produced by combustion, Holt (*21*) has designed a capillary trap which produces a 4-mm. pressure reading per microgram of carbon.

The Fisher Award Lecture of Van Slyke (*58*) gives an excellent review of the status of the wet-combustion method for carbon in various materials, for radioactive carbon, and for other elements precipitated with organic reagents.

NITROGEN

McKenzie and Wallace (*33*) have probably come closer to the optimum conditions for the Kjeldahl digestion than any workers reporting to date. They determined the optimum temperature to be between 380° and 390° C. and obtained that temperature by using 1 gram of potassium sulfate per milliliter of sulfuric acid. Mercuric oxide in sulfuric acid was the catalyst, addition of hydrogen peroxide was unnecessary, and digestion for 15 minutes after clearing was sufficient. A fixed time of boil would be preferable to 15 minutes after clearing, because some materials do not char. The ammonia was absorbed in 2% rather than the 4% boric acid preferred by most analysts. Scandrett (*43*) used as his steam source the superheated steam supplied by the laboratory steam line rather than the customary steam generator. This readily available source, although seldom used, was easy to control, safe, and satisfactory despite the introduction of rust particles into the distillation apparatus.

The sealed-tube method of White and Long had given low results for certain nitro compounds, but Baker (*4*) reported that good results could be obtained if 50 mg. of thiosalicylic acid or glucose were added to the digest. Grunbaum, Kirk, Green, and Koch (*19*) reported a critical study of the sealed-tube technique, determining the temperature effect on the stability of the ammonia and the effect of the amount of sulfuric acid and the addition of water on ammonia stability. Their study covered both the milligram and microgram ranges.

A new "wrinkle" in indicators proposed by Sher (*48*) was a two-step or signaling indicator consisting of bromocresol green, new coccine, and *p*-nitrophenol dissolved in the boric acid absorbing solution. He stressed the point, which is sometimes overlooked, that the solution before titration should always be adjusted to a fixed volume.

A simple colorimetric method for 1 to 20 γ of nitrogen was proposed by Boissonnas and Haselbach (*9*). After digestion with sulfuric acid the digest was neutralized with a lithium hydroxide-acetate solution in 20% Cellosolve, ninhydrin hydrindantin reagent was added, the solution was heated, and the color was measured.

The Dumas method frequently gives low results for certain types of heterocyclic compounds because of retention of nitrogen in the refractory chars formed during analysis. Two groups of analysts, Swift and Morton (*55*) and Parks and others (*42*), described methods for recovering this nitrogen by burning the char with oxygen after the usual combustion in carbon dioxide. Swift and Morton used cylinder oxygen and the other group used electrolytically generated oxygen and the nickel–nickel oxide tube filling of Kirsten. In addition to the emphasis on method for refractory materials, the trend is toward more rapid Dumas-type methods. Swift (*54*) described another such method which required less than 30 minutes per determination and used a carbon dioxide flow rate of 15 to 16 ml. per minute. In a collaborative study reported by this reviewer (*37*) it was noted that more analysts in this country were shifting to the Shelberg and Zimmermann methods and that these procedures gave results as good as or better than those obtained by the more conventional procedures. Kuck and Altieri (*27*) extended the Dumas method to permit analysis of samples liberating less than 0.2 ml. of nitrogen. To do this a nitrometer with a capacity of 0.2 ml. was designed and calibrated in microliters and samples were weighed on a Garner quartz fiber balance. With 0.4-mg. samples the standard error of estimate was ±0.28% nitrogen.

SULFUR

A new direct titrimetric method for sulfate applicable on the semimicro scale was proposed by Fritz and Freeland (*17*). Titration was with standard barium solution using alizarin red S as indicator and was carried out in 30 to 40% alcohol. Equilibrium was quickly attained, permitting a more rapid titration than with sodium rhodizonate indicator, but the coprecipitation errors were greater than with the gravimetric method. When interfering anions were absent, removal with an ion exchange column so improved the results that they were as good as those obtained by the gravimetric procedure. An indirect titrimetric method, described by Wilson, Pearson, and Fitzgerald (*60*), determined the excess barium ion after barium sulfate precipitation by titration with 0.02N (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid, EDTA). These workers used ceria or alumina as catalyst in the combustion and found it to be unaffected by chlorine. Of the indirect methods, this one seems promising because of its simplicity and the probability that coprecipitation will be less because precipitation and titration take place in a very dilute solution.

The silver absorbent method was investigated further by Kuck and Grim (*30*) and Vecera (*59*). Vecera recommended the use of a tube filled with silver shavings because this prevents mechanical loss of the silver sulfate and permits easy extraction, should halogens be present in the material being analyzed. Kuck and Grim again extended the method to decimilligram samples using the Garner balance and found that as little as about 50 γ of sulfur could be determined satisfactorily. For materials containing alkali and alkaline earth metals, Sirotenko (*49*) recommended fusion of the sulfate residue in the combustion boat with boron trioxide, thereby liberating the sulfur trioxide.

Electron microscope studies by Schulek, Pungor, and Guba (*47*) showed that the addition of ammonium chloride at the time the barium sulfate was precipitated changed the morphology of the precipitate and facilitated its filtration. A collaborative study (*39*) showed that the barium sulfate precipitate obtained in the AOAC Carius and catalytic combustion methods need not be subjected to a second washing and ignition procedure.

Anderson (*2*) described a spectrophotometric method for determining microgram quantities of sulfur, in which the sulfur was converted to sulfate, benzidine sulfate was precipitated, and the precipitate was dissolved in hydrochloric acid, made to volume, and measured spectrophotometrically. Ignition of organic substances with magnesium powder was proposed by Schöniger (*44*), who then determined the sulfur iodometrically after distillation of the sulfide by Zimmermann's procedure. The same ignition procedure was also used for chlorine and bromine compounds with the argentometric measurement of Kainz and Resch.

HALOGENS

The Pregl combustion technique followed by a Northrop potentiometric titration was used by Kuck, Daugherty, and Batdorf (*29*) to determine chlorine in small samples. Use of the silver–silver oxalate reference cell with a silver electrode eliminated the need for graphic location of the equivalence point, since the presence of chloride ion displaced the electrochemical equilibrium. The titration merely involved the addition of standard silver nitrate solution until the equilibrium was restored. Thomas (*57*) recommended the simple titration with mercuric nitrate using diphenylcarbazone–bromophenol blue mixed indicator for determining chlorides in aqueous solution.

Chateau (*10*) described an interesting procedure for determining both bromine and iodine even when present with chlorine. The bromates and iodates were formed by oxidation with sodium hypochlorite at pH 5.5 and the excess oxidizer was reduced by potassium formate. At pH 4.1, potassium iodide reduced only the iodate and the liberated iodine was titrated with standard thiosulfate solution. Addition of sulfuric acid to the bromate-iodide solution liberated six atoms of iodine per initial bromine and this was again titrated with thiosulfate. If the ratios of the two halogens were not greater than 200 to 1, reasonably good results were obtained. The method can be applied to silver halides if the halides are put in solution with hydroxylamine in basic solution. The spectrophotometric method of Hunter and Goldspink (*22*) permitted determination of less than microgram quantities of bromine. To do this two quantitative conversions were necessary: first, that of bromide to bromate by hypochlorite oxidation and, second, formation of tetrabromorosaniline by the bromine liberated through the reaction of the bromate with added bromide.

Belcher and coworkers (*5, 6*) reported methods for fluorine and for the other three halogens in fluorine-containing compounds using the nickel bomb method with sodium or potassium. Each halogen was determined separately by accepted titrimetric procedures. Clark and Rees (*11*) also proposed a method for determining all four halogens based on combustion in oxygen. Fluorine was separated from the other halogens by making the latter react with silver. A mathematical calculation was necessary to obtain complete differentiation.

The silver–amalgamated silver electrode system of Clark was adapted to the microdetermination of chlorine, bromine, and iodine by Cogbill and Kirkland (*12*). They stressed the ease of construction of small electrodes and their sensitivity in very dilute solutions. A novel combustion procedure for relatively nonvolatile materials was described by Schöniger (*45*). The sample was burned in a piece of ash-free filter paper in an oxygen atmosphere in an Erlenmeyer flask and the combustion products were absorbed and titrated.

OXYGEN

Oita and Conway (*40*) described a modification of the Schutze-Unterzaucher method for oxygen, which eliminated the need for an 1120° C. furnace. They replaced the carbon filling with one composed of 50% platinized carbon and found that a temperature of 900° C. was sufficient to convert all oxygen to carbon monoxide. They also introduced a copper roll in the heated portion of the tube to remove sulfur and found that with this combination the blanks were usually negligible. Oliver (*41*) confirmed the findings of Oita and Conway and also described methods for the preparation of the carbon-platinum catalyst, copper gauze, and iodine pentoxide. Four different modifications of the Unterzaucher method were described by Jones (*24*). Two procedures for determining carbon dioxide, one gravimetric and one volumetric, were used, iodine was absorbed on sodium thiosulfate crystals, and in the fourth pyrolytic hydrogen was removed by diffusion through palladium, followed by the usual liberation and thiosulfate titration of the iodine.

MISCELLANEOUS ELEMENTS

Bernhart and Wreath (*7*) described a modified spectrophotometric method for determining orthophosphate which is not affected by moderate amounts of pyro-, meta-, or polyphosphates. Color is developed in a solution containing sulfuric acid and at least 25% acetone, and the method is applicable to solutions obtained after either acid digestion or alkaline fusion of organic materials. Di Bacco (*15*) proposed a molybdenum blue procedure in which sulfuric acid was used but not acetone, and applied a similar method to the determination of arsenic. Lévy (*31, 32*) also described methods for both phosphorus and arsenic, but used a potentiometric titration procedure in which silver nitrate was added in excess of that needed to precipitate the anion and the excess silver was titrated with a standard chloride solution. In both procedures an empirical correction was necessary. The iodometric determination of arsenic in organic materials on a semimicro scale, proposed by Bähr, Bieling, and Thiele (*3*), required sulfuric acid digestion in a Kjeldahl flask followed by permanganate and perhydrol oxidation. The iodine liberated by the arsenate was determined by thiosulfate titration. Martin (*34*) increased the specificity of the colorimetric boron method using chromotrope 2B by replacing the concentrated sulfuric acid with a mixture of sulfuric and acetic acids plus acetic anhydride.

FUNCTIONAL GROUPS

Heron and coworkers (*20*) made a critical study of the methoxyl and ethoxyl group determinations and found the main source of error to be the thiosulfate in the wash solution. This was replaced by 25% sodium acetate with a considerable improvement in results, especially when ethoxyl groups were determined. In two collaborative studies reported by Steyermark (*50, 53*), analysts using the Clark method obtained better results than those using the Elek procedure. Methoxyl and ethoxyl ethers and esters were determined. However, when all analysts used a modified Clark procedure the results were not sufficiently accurate or precise to recommend the adoption of the method as an official AOAC procedure. A further modification of the method following the suggestion of Heron and others (*20*) did lead to good results in collaborative work concluded this year but not yet published. To determine methoxyl in the presence of ethoxyl, Gran (*18*) absorbed the iodides in trimethylamine dissolved in isopropyl alcohol. Tetramethylammonium iodide precipitated quantitatively on standing overnight. Mathers and Pro (*35*) used an entirely different approach. Methoxyl was cleaved to methanol and oxidized to formaldehyde, and the latter was determined spectrophotometrically by the chromotropic acid method. The method gave good results and is specific for the methoxyl group.

Schöniger, Lieb, and El Din Ibrahim (*46*) used the Parnas-Wagner Kjeldahl distillation apparatus to steam-distill the acetic acid formed either by oxidation of C-methylated substances or by saponification of acetyl compounds. Only 10 minutes were required for a distillation, titration was with standard alkali, and the results were good. In the collaborative study reported by Steyermark (*53*), neither the Roth-Kuhn nor the Elek and Harte method consistently gave satisfactory results. Tani and Nara (*56*) avoided distillation of the acetic acid by passing the solution after saponification through a column of cation exchange resin in the hydrogen cycle and titrating the effluent with standard base.

STANDARDIZATION

Official methods for the determination of carbon and hydrogen, nitrogen by the Kjeldahl method, sulfur, chlorine, and bromine have been adopted by the Association of Official Agricultural Chemists (*38*). A method for methoxyl and ethoxyl determination has been recommended for adoption and a preliminary study of methods for phosphorus has been completed, but neither has been published.

The Committee on Microchemical Apparatus of the Division of Analytical Chemistry, AMERICAN CHEMICAL SOCIETY, has published recommended specifications for numerous pieces of weighing and drying apparatus (*51*). The committee has also drawn up specifications for a modified Clark alkoxyl apparatus (*52*).

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REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Statistical Methods in Chemistry

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CONSIDERABLE progress, both in theory and in the number of applications, has been made since the last review of statistics in chemistry by Hader and Youden (*89*), in this journal. Because the authors felt that the present review should concentrate on general developments rather than present a comprehensive bibliography, many of the papers described here in detail have been selected because they are convenient illustrations of the ideas and methods discussed in the text. Inclusion or omission of a particular paper in this bibliography should not be considered as an attempt to judge its merit. Furthermore, while an effort was made to cite as many articles as possible from the chemical literature, it has been indispensable, in view of the intended nature of this review, to refer frequently to statistical journals. In doing this, priority was given to those articles in which the underlying ideas, rather than mathematical proofs, are presented.

The selection of topics for discussion is necessarily somewhat arbitrary and subjective. It is based, not so much on logical order, as on a consideration of the current trends in statistical methodology. Here again, as in the listing of individual papers, completeness has not been attempted. Thus, a variety of topics which can legitimately be considered as statistical and useful in some chemical applications were nevertheless omitted. The topics that are considered deal, broadly speaking, with the drawing of inferences from measurements obtained in planned experiments.

ACCURACY AND PRECISION OF METHODS AND EQUIPMENT

The concepts of accuracy and precision are examined in detail by Eisenhart (*63*), who proposes definitions based on an operational viewpoint. With regard to the definition of precision, Eisenhart points out that the reproducibility of a method depends on the conditions under which the repeated measurements are carried out. If, in the sequence of repeated measurements, more than one instrument, or operator, or laboratory is used, the variability due to these factors will affect the precision of the method. The operational definition of precision would therefore specify the exact nature of the sequence of repeated measurements.

In regard to accuracy, Eisenhart remarks that unbiasedness—i.e., complete absence of systematic errors—does not yield its full benefit unless enough replicate measurements are made to reduce the standard error of their average to a negligible value. Thus, in cases where only a few replicate measurements are averaged, a biased but reproducible method is often preferable to one having no systematic errors but less reproducibility.

Youden in an earlier paper (*213*) and in his book (*216*) described a method involving linear regression techniques for resolving relative and constant type errors in studying the accuracy of a procedure, using the "standard error of estimate" as a measure of precision. Recently, this approach has been used in the study of test methods (*92, 115, 125*). Linnig, Mandel, and Peterson (*125*) describe a plan for studying accuracy and precision based on this concept and on analysis of variance techniques. A flexible relationship between the concepts of accuracy and precision as applied to a given analytical procedure is indicated along with types of factors affecting each. Lark (*115*) has indicated the necessity in studies of this type involving regression analysis of using joint tests of significance on both the slope and intercept in order to allow for the interdependence of their errors in making "confidence statements." Mood's book (*147*) constitutes an excellent source on joint confidence tests, including computational details. This subject is of great general importance and is dealt with in more detail in the section on the interpretation of data. Mandel and Stiehler (*137*) have introduced the concept of sensitivity to compare the merits of alternative methods of test. Sensitivity, as defined by these authors, relates to the ability of a method of measurement to detect small differences in the property under study and involves, besides the standard deviation of the measurement, the relationship between the property and the measured quantity. The use of various recently developed statistical techniques for the study of analytical procedures is effectively described in two expository articles by Youden (*214*) and Box (*20*).

In methods requiring standard materials, the difficulties due to chemical instability, such as in the case of photographic materials, have been dealt with by West (*207*) by means of statistical techniques. The precision of results of microanalyses is appreciably influenced by the observer's unconscious preferences for certain fractions in estimating the last place on the pointer scale of the balance. Gysel (*88*) has shown that these preferences differ from person to person but are remarkably constant for a given individual. This phenomenon may well deserve consideration in the calibration and use of other types of equipment. A number of articles (*33, 52, 66, 74, 101, 107, 116, 138, 160, 163–165, 180, 184, 210*) involve the study and calibration of equipment of various types. Many articles have appeared, covering a wide range of problems involving accuracy and precision (*6, 7, 15, 19, 26, 31, 32, 42, 61, 67, 75–77, 81, 92, 96, 100, 102, 114, 118, 122, 127, 133, 142–144, 146, 166, 169, 178, 179, 182, 183, 185, 187, 189, 198, 201, 218*).

DESIGN OF EXPERIMENTS

Numerous applications of the principles and procedures of statistical design are recorded in the recent literature, with particular emphasis on factorial designs and latin squares (*1, 8, 22, 23, 38, 45, 51, 56, 79, 82, 95, 103, 112, 120, 139, 145, 150, 151, 162, 166, 167, 171, 174, 175, 182, 197, 203, 212*). At the same time, new trends are emerging, which are of particular interest in the physical sciences. A number of these are discussed below.

Fractional factorial designs are a class of designs that have been considered of particular usefulness in preliminary or exploratory investigations of the effects of many controlled variables on a measured quantity. In these designs, a selection is made of the factorial combinations, instead of the complete coverage required in full factorials. The selection is such that only effects that are deemed of secondary importance (generally high-order interactions) are sacrificed. A lucid description of these designs is given in an expository article by Brownlee (*28*). Other articles of interest have appeared in the literature (*43, 109, 196*). The texts by Davies (*47*), Bennett and Franklin (*16*), and Cochran and Cox (*37*) contain well-written chapters dealing with fractional factorials. Briefer accounts are given by Brownlee (*29*), Wilson (*211*), and Youden (*216*).

There has been a greater utilization of a class of designs useful in scheduling experiments, in the sense of making the most efficient use of available equipment, personnel, time, and similar factors. Compensation for the systematic errors introduced by variation of these factors is accomplished through the device of partitioning the measurements into statistical "blocks." A block is a group of measurements characterized by some constancy of experimental conditions, such as a single day, the same oven, or the same batch, or sheet, or roll of material. The application of block designs to chemical experimentation is by now well established.

In "randomized block designs," treated in a number of texts (*5, 16, 29, 37, 47, 54, 147*), all the treatments occur in each block in a randomized order, the term "treatments" referring to the categories that are to be compared in the test—e.g., different materials, different concentrations, different temperatures, different solvents, or different brands of a commercial product. Further progress was accomplished through the introduction of "incomplete blocks," which are required in situations where the blocks are too small to include one measurement for each treatment. The bias due to variations among blocks is eliminated in these designs by proper overlapping of some of the treatments in all blocks. How this is accomplished is clearly described in nonmathematical terms by Youden (*216*), and some applications are described in the literature (*82, 94, 121, 193*). Certain incomplete block designs have the maximum possible symmetry, in which case they are called "balanced" (*5, 16, 29, 37, 47, 216*) and lead to a constant precision for all pairwise comparisons of treatments. However, in order to achieve balance, it is often necessary to include a rather large number of measurements for each treatment. Designs that allow for proper scheduling with fewer measurements per treatment are now available and are known as "partially balanced incomplete blocks" (*37*). In applications to chemistry, the disadvantage of having slightly different precisions for the various treatment comparisons appears of secondary importance because of the relatively high precision of measurements in the physical sciences and is generally more than compensated for by the economy of these designs. An interesting class of partially balanced incomplete block designs, known as "chain blocks," has been introduced by Youden and Connor (*217*). Mandel (*135*) describes a flexible class of designs, related to the chain blocks and requiring no more than two measurements per treatment. An application of the latter is described by Rothman and coworkers (*172*). An elementary discussion of chain block designs and their usefulness in scheduling experiments is available (*149*).

Balanced incomplete block designs are tabulated in a number of places (*37, 47, 70*), and a compilation of an important class of partially balanced incomplete block designs, with instructions for analysis, has been made available by Bose, Clatworthy, and Shrikhande (*18*). Designs based on blocks of size two—i.e., containing only two measurements per block—have been extensively studied, and an up-to-date account is given by Clatworthy (*36*). They have been used by Youden (*215*) for the detection and measurement of instrumental drift. Page (*152*) explores their possible use for the calibration of meter bars.

Sequential designs are schemes of stepwise experimentation in which each step depends on the results of the previous experiments. Gore (*78*) describes a sequential plan based on 2 × 2 latin squares. Box and his coworkers (*21, 24*) have developed extensive schemes of sequential experimentation for the exploration of "response surfaces," a name given by these authors to the result of a measurement such as yield, considered as a function of a number of controlled variables (geometrically, a surface in a higher-dimensional space). Box's efforts are aimed more specifically at the determination of conditions leading to a maximum of the measured quantity. Good expository discussions are given by Box (*20*) and by Read (*161*), and a comprehensive chapter is included by Davies (*47*).

The problem of selecting from among several populations the one that is optimum with respect to some property has also been treated by Bechhofer (*10, 12, 13*), with particular reference to the determination of the minimum number of measurements that are needed to make this selection with a risk of error not to exceed a preassigned value. The author describes his method in an easily read expository article (*11*). The techniques introduced by Bechhofer are also noteworthy in another respect discussed in the section on the interpretation of data.

INTERPRETATION OF EXPERIMENTAL RESULTS

Most catalogued statistical designs are such that the "least squares" solutions (*5, 48, 54, 147*) for the effects of factors or blocks or for the comparisons among treatments are obtained with very little arithmetic. They also lend themselves readily to treatment by the "analysis of variance" technique (*5, 16, 29, 48, 54, 147, 216*), which provides an estimate of experimental error and serves as a guide in ascertaining the significance of the effects of the various factors on the measured properties. It has been recognized, however, that analysis of variance is generally only a first step in the interpretation of data.

Eisenhart (*62*) has pointed out that the interpretation of the analysis of variance depends on the nature of the factors involved. Thus, the factor "day of experimentation" is of a random type, whereas a factor such as "catalyst" is termed "fixed." Eisenhart defines certain "models" based on the nature of the factors in the experiment. The distinction is particularly important when it is desired to derive from the mean squares measures known as "components of variance" to estimate the effects of certain factors, since the steps in the calculation depend on the model involved. The present state of knowledge in this field is reviewed in a comprehensive article by Crump (*41*). Pertinent discussions are given in the books by Mood (*147*), Anderson and Bancroft (*5*), and Bennett and Franklin (*16*). Some applications of components of variance appear in the literature (*119, 125, 165*).

Hamaker (*93*), in an expository article dealing with industrial applications, emphasizes the usefulness of a technique combining analysis of variance and regression analysis, for factors susceptible of continuous variation on a scale, such as temperature or concentration. By this technique, the mean square corresponding to the effect of such a factor is decomposed into components relating to linear, quadratic, cubic, and, if necessary, higher order terms. Thus, it may be judged whether, within the limits of experimental error, the relation between the measured quantity and this factor is linear, quadratic, etc. This technique is also described by Brownlee (*29*) and in greater detail by Davies (*47*).

A number of recent investigations are of particular interest from the viewpoint of the validity and the scope of inferences drawn from experiments. They emphasize the efforts made in recent years to clarify the basic principles underlying the application of statistics. These developments are already reflected in some applications and are likely to affect to an even greater extent future work in applied statistics. It is natural that a variety of approaches have been proposed, differing in fundamental concepts. In practice, however, this diversity of viewpoints permits the experimenter to select that approach which he considers most appropriate for the problem at hand.

Especially noteworthy is the fundamental work of Scheffé (*176*) and Tukey (*199*), by which the heretofore unsolved problem of the reliability of "multiple comparisons" receives a rigorous solution. The difficulty resulted from the effect of the interdependence of conclusions drawn from the same set of data on the joint reliability of these conclusions. In solving the problem, Scheffé and Tukey have taken the viewpoint that probability statements concerning the reliability of conclusions drawn from a set of data should concern the experiment as a whole—i.e., the totality of all conclusions drawn from it—rather than each conclusion individually. An important practical aspect of this problem deals with the construction of confidence intervals (*16, 91, 147, 211*)—i.e., intervals within which the true values that are sought can be said to lie with a given probability. If the intervals corresponding to these various values are not statistically independent it may sometimes be advisable to evaluate their reliability jointly rather than separately for each interval. This joint procedure leads to ellipsoidal confidence regions from which the individual confidence intervals can be derived, and the probability measuring the confidence of the conclusions (the "confidence coefficient") applies to the entire region, rather than to individual intervals. An excellent and not too mathematical account of the problem is given by Durand (*59*). This author points out that joint confidence regions are more particularly useful when a multiplicity of conclusions are likely to be drawn from the experiment and the choice of comparisons must be deferred until after the data are obtained.

Duncan (*57*), in dealing with the problem of grouping a set of treatments on the basis of their observed averages, uses a different approach to the problem of multiple comparisons. If a significance level α is adopted—e.g., $\alpha = 0.05$—Duncan associates with each comparison of treatments a probability of error α', such that $1 - \alpha' = (1 - \alpha)^{p-1}$, where p is the number of treatments involved in the comparison. It is seen that only comparisons involving two treatments ($p = 2$) are made at the specified significance level, α. As the number of treatments involved in a comparison increases, the level α' increases and the reliability of the comparison decreases. Thus, Duncan's method is less stringent than Scheffé's, which requires that the probability of even a single error in the totality of all comparisons among treatments be at most α. An application of Duncan's method to a problem of taste testing has been described (*58*).

A striking illustration of the importance of the interdependence of confidence statements derived from the same data is given by Lark (*115*). In a situation requiring a decision as to whether an observed set of data fits a straight line with slope equal to unity and intercept equal to zero, Lark shows that separate 95% confidence intervals for the slope and intercept lead to an obviously erroneous conclusion, while the conclusions based on a joint confidence ellipse for slope and intercept are entirely reasonable.

Joint confidence intervals arise also in experiments involving the simultaneous measurement of several properties. Daniel and Riblett (*43*) evaluate the effects of several controlled variables in the preparation of a catalyst by means of measurements of its activity and selectivity. They apply the results of multivariate analysis (*147*) for the construction of a joint confidence region, in this case an ellipse in a plane whose coordinates are activity and selectivity. The joint analysis shows that one of the factors, which on the basis of separate tests of significance for selectivity and activity was apparently without effect, is actually clearly significant, while two other factors, which on the basis of the individual tests appeared definitely significant, are actually of doubtful significance.

Joint confidence regions are also appropriate in the quality control of materials or consumers' products. Beall and Pascoe (*9*) present an interesting procedure for the quality control of paper based on two properties, such as basis weight and burst strength. Their method, which also involves multivariate analysis, permits the elimination of material that is deficient in either one or in both properties, with due consideration of the correlation that exists between the two tests. A somewhat different application of multivariate analysis is made by Fisher, Hansen, and Norton (*68*) in the simultaneous analysis of glucose and galactose by a spectrocolorimetric method. Observations are made at two wave lengths and the results are used to solve two simultaneous quadratic equations in the two unknowns. The absorbance at each wave length can be considered *as a function* of the concentrations of glucose and galactose. Tested separately, these functions for the two wave lengths appear to be linear, while a joint analysis reveals that quadratic terms must be included. The reason for the inadequacy of separate statistical tests for the two wave lengths is the high correlation between the errors of the two measurements.

The usefulness of experimental results depends to a large extent on their precision, which, in turn, is a function of the measuring process. Considerations of precision, accuracy, and sensitivity, discussed in an earlier section, will generally be used to select the better measuring process from among alternate choices; but even after a particular method of measurement has been selected, the precision of the final results still depends on the number of replicate measurements and on the statistical procedure used in the processing of the data. One way of specifying the precision of the final results is to require that effects of a given, preassigned magnitude shall be statistically significant and therefore detected. The ability of a statistical significance test or estimation process to satisfy such a requirement is expressed by the "power" concept, described in a number of texts (*5, 54, 91, 147, 211*). Greater power is characterized by shorter confidence intervals. Clearly, an increase in the number of replicate measurements leads to higher power. Conversely, decisions regarding the number of determinations to be made, or the number of samples to be tested, can be made on the basis of power requirements. In the chemical literature, a number of papers describe applications of the power concept (*71, 125, 134, 136, 153, 158*).

Power considerations form an integral part of the sequential test procedures developed by Wald, whose book (*202*) is an excellent source, even for nonmathematical readers. Shorter accounts are given by Mood (*147*) and Davies (*47*). Churchman (*35*), in a paper already mentioned in a previous review (*206*), and Kasagi (*104*) apply sequential methods to decide between two possible empirical formulas for a chemical compound. In Churchman's paper, the decision is based on the observed percentages of bromine and sulfur. Here, the power concept relates to the risk of choosing the incorrect formula. As successive samples are analyzed, the combined results, though affected by experimental error, tend toward the percentage values for the correct formula. The advantage of the sequential procedure results from the likelihood that this tendency will be conclusive, in terms of the tolerated risk, in relatively few determinations. Theory shows that the saving in the number of determinations is often of the order of 50% as compared to experiments having a predetermined number of determinations and the same risk value.

The theory developed by Bechhofer (*11*), which has already been mentioned, is also noteworthy in that it introduces considerations of power, in the form of the "smallest difference worth detecting," at the outset rather than as a secondary consideration. The choice of the "smallest difference worth detecting" is considered by Bechhofer as an extra-statistical decision to be made by the experimenter, possibly on the basis of economic considerations. Somerville (*188*), in a theoretical investigation dealing with the problem of selecting, from among several categories, that which is optimum in some specified respect, incorporates into the statistical procedure the cost of sampling and the economic losses resulting from an erroneous selection. The work of both Bechhofer and Somerville is based on a "decision-theoretic" approach, a recent development in which statistical procedures are viewed as rules for making decisions, the value of each decision being determined by its economic consequences. A popular exposition of decision theory is given by Bross (*27*). This approach has been found most useful in industrial applications. In more fundamental work, different approaches have been used. Thus, in a study of the accuracy and precision of an analytical method, the power of the test has been related to the standard deviation of the analytical method (*125*). Fisher (*69*), in a stimulating recent article of a philosophical nature, opposes the injection of cost considerations or of decision-making in applications to fundamental research and concludes that, in such applications, "We aim, in fact, at methods of inference which should be equally convincing to all rational minds, irrespective of any intentions they may have in utilizing the knowledge inferred."

Building "power" requirements, whether based on economic or other considerations, into the design of an experiment appears to be a first, though significant, step in the growing trend of tailoring each design exactly to the particular requirements of the experiment, with only selective use of catalogued schemes. This is particularly true for investigations that have progressed well beyond the exploratory stages and are concerned with the study of detailed relationships, such as those discussed in the following section.

STUDY OF CHEMICAL REACTIONS AND LAWS

A few papers have appeared in the literature concerning the use of statistical methods in the study of chemical reactions and laws. Box and Youle (*25*) have related the parameters in the equations of a fitted surface to parameters describing the kinetics of the reaction. The surface results from the measurement of the end product of the reaction obtained under different conditions of temperature, time, or concentration of reactants. The technique of "exploration and exploitation of response surfaces" has been mentioned in the section dealing with the design of experiments. Another paper, mathematical and theoretical in nature, by Singer (*186*) investigates the factors causing irreproducibility in chemical reactions. For example, chain reactions with a very slow rate of initiation followed by a very rapid chain propagation are irreproducible because of the very nature of the mechanism involved. A method is proposed for distinguishing between this type of irreproducibility and that which is caused by the introduction of extraneous impurities or other similar factors. Grohskopf (*84*) discusses the use of factorial designs in the study of chemical reactions, stressing the importance of interactions, often dismissed by investigators, for the detection and measurement of physical and chemical relationships. He suggests the use of factorials for the study of reaction velocities.

Liebhafsky, Pfeiffer, and Zemany (*123*) verify experimentally that x-ray emission is a random process with a standard deviation equal to the square root of the count per unit of time. This standard deviation increases, however, when operating conditions are not ideal. The theoretical standard deviation may therefore be used as a control in x-ray emission spectrography. Linnig and coworkers (*125*) observe that variation in the quantity of end product in equilibrium reactions may result in a curvilinear relationship between material found and added. The presence of curvature can be detected and measured by statistical means. Whitman and Whitney (*208*) have used regression analysis to determine the order of a reaction. These and some other similar papers (*39, 40, 44, 113, 131, 140, 168*) represent a step in the direction of the practical application of statistics to the study of fundamental chemical problems. But the possibilities of relating statistically derived parameters to natural laws are only now being exploited. The value of this approach over the classical approach is due to a great extent to the statistical practice of varying a number of factors at a time, thus not losing sight of important interrelationships between these factors.

MISCELLANEOUS APPLICATIONS

Nonquantitative or semiquantitative data, such as arise in the taste-testing of food (*34, 50, 57, 83, 98, 99, 117, 121, 141, 155, 212*), have effectively been treated by statistical techniques known as "nonparametric" or "distribution-free." As these methods are based entirely on the ranking of the observations, they require no assumptions regarding the underlying distribution of the data and have therefore also been very valuable in the analysis of quantitative measurements when the assumptions underlying other statistical methods are of questionable validity. Among the most useful nonparametric tests, of wide applicability, are the sign test (*55*) and the rank-correlation coefficient. These, as well as other nonparametric methods, are described in books by Mood (*147*), Dixon and Massey (*54*), and Bennett and Franklin (*16*). Especially noteworthy is the little book by Moroney (*148*), in which the rationale and practical application of many nonparametric tests are lucidly described without mathematical developments. An interesting example of the use of rank-correlation methods is provided by Schmidt (*180*) in a study of the interchangeability of spectrophotometric absorption cells, with comments on other applications. Convenient tables for the use of the rank-correlation coefficient are given by Litchfield and Wilcoxon (*126*).

The old and vexing problem of the "rejection of outlying observations" has received recent discussion by Dixon (*53*), Proschan (*157*), King (*111*), Grubbs (*85*), and Blaedel, Meloche, and Ramsay (*17*).

Kase (*105, 106*) has applied the theory of "extreme values" to problems in polymer research and rubber technology. This theory deals with observations which, by their very nature, lie in the tail end of a frequency distribution—e.g., the largest flood a bridge is intended to withstand, or the strength of the weakest fiber in a rubber tensile specimen. A very readable and comprehensive treatment is given by Gumbel (*86*) and a newer method of analysis by Lieblein (*124*). These authors have also compiled a list of recent applications in a short, expository article (*87*). Practical applications are discussed by Kimball in a recent article (*110*).

A number of papers (*4, 177, 205*) deal with the problem of devising efficient designs for large scale interlaboratory studies of test methods or equipment and with methods of analysis of the resulting data. An appreciable number of interlaboratory tests are described in the literature (*30, 52, 60, 67, 114, 128, 159, 165, 178, 181, 190, 191*).

The problem of estimating the useful life of manufactured products has been the object of statistical studies, many of which are in the form of unpublished technical reports. Among the published work, there are papers by Weibull (*204*), Freudenthal and Gumbel (*72, 73*), McClintock (*129, 130*), Epstein and Sobel (*64, 65*), and McElwee (*132*). The American Society for Testing Materials has devoted two special technical publications to the subject (*2, 3*). Davis (*49*) has written a paper of an expository type, including the analysis of 13 systems encompassing a wide range of applications.

The increasing availability of high-speed computing equipment to many research workers will undoubtedly be reflected in a more widespread use of multiple regression methods for the analysis of large amounts of data. The application of Box's techniques (*20, 47, 161*), mentioned in an earlier section, often involves extensive computations. Electronic calculators have been used in a study of infrared absorption and emission spectra by Plyler, Blaine, and Tidwell (*156*) and in a factorial experiment described by Rowell (*173*). A bibliography of recent articles and books dealing with computing machines, including also other statistical and mathematical references, is given by Rose, Johnson, and Heiny (*170*).

BOOKS, TABLES, AND ABSTRACTS

The following is a partial listing of the numerous books, that have recently appeared, which cover statistical theory on various levels of mathematical difficulty and with varying emphasis on applications.

The basic theory is well covered by Mood (*147*), Hoel (*97*), Hald (*91*), and Anderson and Bancroft (*5*). More elementary expositions of statistical theory are given by Wilks (*209*), Tippett (*194*), and Dixon and Massey (*54*).

Among the books written more specifically for chemists, that by Bennett and Franklin (*16*) is the most comprehensive, while the books by Youden (*216*), Brownlee (*29*), and Davies (*48*) provide good introductory treatments. Other books in this area have been written by Tippett (*195*), Villars (*200*), Gore (*80*), David (*46*), and Beers (*14*).

A book deserving special mention, because of its unique approach, is that written by Wilson (*211*). It contains many interesting discussions on the rational basis and the practice of the statistical method.

The design of experiments has been comprehensively treated by Kempthorne (*108*), with considerable mathematical detail. An excellent practical book on the design and analysis of experiments has appeared under the editorship of Davies (*47*). Cochran and Cox (*38*) provide a comprehensive catalog of designs, with explanatory chapters on their characteristics and analysis. Finally, a casual, yet clear and detailed exposition of a large number of useful statistical techniques is contained in the book written by Moroney (*148*).

Statistical tables are appended to most statistical texts. A particularly complete set is that found in the book by Dixon and Massey (*54*). Hald has issued, in conjunction with his book, a compilation of many useful tables (*90*). Fisher and Yates' tables (*70*) have served statisticians and research workers for many years. A newly revised and very comprehensive version of the well known *Biometrika* tables, with detailed explanatory notes, has been made available by Pearson and Hartley (*154*).

Many articles dealing with statistical methods appear in the chemical and related literatures. In addition, such journals as *Biometrics*, *Industrial Quality Control*, the *Journal of the American Statistical Association*, the *Journal of the Royal Statistical Society, Series B*, and *Applied Statistics* occasionally contain articles useful in chemical work.

A column devoted to discussions on statistical design appears on a bimonthly basis in *Industrial and Engineering Chemistry*, under the editorship of W. J. Youden.

Abstracts of statistical articles, both theoretical and applied, are regularly compiled by G. E. Nicholson, Jr., in the *Journal of the American Statistical Association*, and by Joseph Movshin in *Industrial Quality Control*. A further source is the *International Journal of Abstracts on Statistical Methods in Industry*, edited by G. I. Butterbaugh.

ACKNOWLEDGMENT

It is a pleasure for the authors to acknowledge their indebtedness to Grant Wernimont, author of the 1949 review on statistics in this journal (*206*), for supplying a collection of more than 400 abstracts of references, from which the major portion of papers listed in the present bibliography has been taken.

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**REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS**

Characterization of Organic Compounds

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THE term "characterization," according to Peck and Gale, the authors of the previous review on this subject (*33*), involves the following steps: establishment of purity of sample; determination of physical properties; determination of elementary composition, functional groups, and empirical formula; and elucidation of structural formula and spatial relationships.

Craig (*15*) gives the following steps as being involved in a complete structural study of large naturally occurring molecules: isolation and characterization of a single chemical individual, including the accumulation of proof that such an objective has been reached; determination of molecular size; determination of functional groups; degradation to smaller fragments which can be identified with known substances or, if not, which can be characterized and in turn degraded; derivation of the manner in which the known fragments are connected; and synthesis.

A difference in terminology is evident: Peck and Gale's characterization means the whole story of qualitative organic analysis, purification techniques, spectroscopy, etc. Craig's seems to imply those techniques useful for distinguishing pure samples.

The reviewer has a preference for Craig's viewpoint, and considers the term characterization to be too broad to be useful unless chemists can agree on a precise meaning.

To escape being limited by the dictionary definition as "a description by a set of characteristics" the reviewer must assume that characterization means the sum of all the steps in both lists above. This review consists of five of the cases of noteworthy work completed from November 1951 to November 1955 on the chemical constitution of some natural products. These cases, which illustrate the steps involved, are: tyrocidine A, which interested the reviewer because its story depends so much upon separative technique; oxytocin, because it is the first account of the structural formula determination and synthesis of an octapeptide hormone; insulin, because it is a Herculean accomplishment; α-lipoic acid, because its story illustrates the chore of a 600,000-fold concentration of a material from a complex natural source and a synthesis which produced the right material but missed giving structural information; and vitamin B_{12}, which is important because it includes the vital work of x-ray crystallographers on the problem before the chemists had finished their part of the job.

The fact that three of the five examples discussed are polypeptides or small proteins is no accident. The reviewer believes that the elucidation of such complex structures had to wait for the development of highly refined separative methods, such as countercurrent distribution by Craig, electrophoresis by Tiselius, paper chromatography by Martin and Synge, and ion exchange by Moore and Stein.

TYROCIDINE A

This antibiotic is produced by *Bacillus brevis* in a mixture called Tyrothricin, which consists of gramicidins and tyrocidines. A concentrate in crude crystalline form was resolved by a 220-stage countercurrent distribution with a mixture of methanol, $0.1N$

hydrochloric acid, benzene, and chloroform (23 to 7 to 20 to 10), into about five components which differed in their amino acid contents (*5*). One of these components, tyrocidine A, was distributed in other systems until no further separation could be obtained. Ultimate analysis showed a composition corresponding approximately to $C_{66}H_{87}N_{13}HCl$ (*5*).

The method of partial substitution with Sanger's (*45*) reagent (1-fluoro-2,4-dinitrobenzene) was next applied. Insufficient reagent was reacted with tyrocidine A, and the product was separated in a 220-tube experiment with same solvents as above. Four well separated peaks resulted:

Band 1 (K = 14.6), unchanged tyrocidine A.
Band 2 (K = 1.908), gave *N*-dinitrophenyl-ornithine on hydrolysis.
Band 3, small, broad peak.
Band 4 (K = 0.198), di-dinitrophenyl derivative, gave *N*-dinitrophenyl-ornithine and *O*-dinitrophenyl-tyrosine.

Knowing that the molecular extinction coefficient at 350 mμ for the dinitrophenyl-primary amino group in any amino acid is fairly constant (*4*), and knowing the absorptivity per milligram of Band 2, Battersby and Craig calculated a molecular weight of 1300 (*6*).

Tyrocidine A was next completely hydrolyzed and the amino acids were separated by countercurrent distribution (*31*). Found: two moles of D-phenylalanine, two moles of ammonia, and one mole each of L-phenylalanine, L-valine, L-tyrosine, L-leucine, L-proline, L-ornithine, L-glutamic acid, and L-aspartic acid per mole of tyrocidine A. The original tyrocidine A contained no —COOH but did show two —$CONH_2$ groups per 1300 grams of sample. A cyclic peptide made from these amino acids would add up to $C_{66}H_{87}O_{13}N_{13}HCl$ = 1306.92, making allowance for the conversions of glutamine to glutamic acid and asparagine to aspartic acid during the hydrolysis.

Partial hydrolysis of tyrocidine A under various conditions, followed by ion exchange separation of acidic, basic, and neutral peptides, followed by paper chromatograms and by complete hydrolyses of the separated peptides led Paladini and Craig (*31*) to postulate the sequence:

Tyr.Val.Orn.Leuc.D-Phe.
·
Glu.Asp.D-Phe.L-Phe.Pro.

for tyrocidine A.

It is noteworthy that both optical isomers of phenylalanine are involved.

OXYTOCIN

The posterior pituitary produces oxytocic (uterine-contracting and milk-ejecting) and pressor hormones. Oxytocic activity is measured by the contractile response of strips of rat or guinea-pig uterine muscle (or by decreases in chicken blood pressure (*14*)).

Potts and Gallagher (*36*) separated the oxytocic and pressor factors by chromatography of a pituitary hormone preparation on a synthetic Zeolite and obtained a noncrystalline oxytocin showing 700 units per milligram of activity.

In 1949 Livermore and du Vigneaud (*24*) investigated distribution methods for separating oxytocin from a sample of Pitocin showing 20 units per milligram. A preliminary distribution between 2-butanol and 2*M* phosphate buffer (pH 6.8) showed that 90% of the original oxytocin activity was found in the butanol washings, while 91% of the inert solids stayed in the phosphate. Then, using successive 53-transfer countercurrent distributions with the solvent pairs 2-butanol–0.05% aqueous acetic acid or 2-butanol–0.01*M* ammonium hydroxide, Livermore and du Vigneaud obtained oxytocin showing 865 units per milligram, an activity which could not be increased by further attempts in purification.

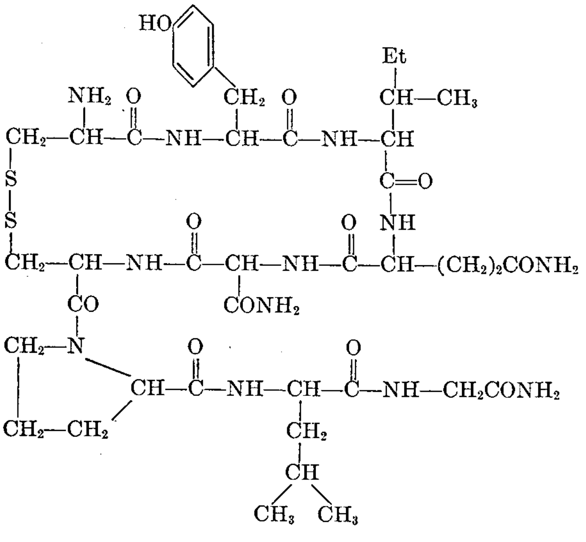
Oxytocin (850 units per milligram) was completely hydrolyzed by Pierce and du Vigneaud (*35*) according to the method of Stein and Moore (*54*), the amino acids were separated by starch-column chromatography (*28*), and the acids were estimated by Moore and Stein's (*29*) ninhydrin color method. The results were: leucine, 1.00; isoleucine, 0.98; tyrosine, 0.72; proline, 0.99; glutamic acid, 1.07; aspartic acid, 1.03; glycine, 1.01; ammonia, 3.46; and cystine, 1.01. A very small amount (0.5%) of phenylalanine was observed; it came from an impurity in this particular starting sample of oxytocin. The low ratio for tyrosine and the high ammonia ratio were later reconciled when it was shown that tyrosine decomposes in part to give ammonia during acid hydrolysis.

Ultimate analyses (*34*) showed: carbon, 50.12%; hydrogen, 6.84%; nitrogen, 16.12%; and sulfur, 6.15%. Theoretical values for a linear polypeptide of the eight amino acid and three amide groups would be carbon, 50.4%; hydrogen, 6.68%; nitrogen, 16.40%; and sulfur, 6.25%.

Performic acid oxidized oxytocin (*30*), splitting the cystinyl to two sulfoalanyl groups. As only one degradation product was found in the reaction mixture, the sulfoalanyl groups must have been attached to the same peptide chain. This would be true only if oxytocin contained cystine in a cyclic structure.

Desulfurization of oxytocin with Raney nickel (*58*) likewise showed the cyclic structure of oxytocin. Only one degradation product was formed, as shown by unsuccessful attempts to find others by countercurrent distribution, paper chromatography, or ion exchange methods. Complete hydrolysis of this single product gave two alanines instead of cystine, plus the other amino acids listed above.

Sanger's technique (*45*) for labeling the original primary amino groups, followed by complete hydrolysis, showed (*18*) that only one free amino group was present in oxytocin on one side of the cystinyl group. The tyrosyl phenolic group formed an *O*-dinitrophenyl ether. The dinitrophenyl labeling of free amino groups in the peptides for partial hydrolysis, followed by complete hydrolysis of each dinitrophenyl-peptide served to elucidate the order of amino acids in their peptides and finally the sequences involved in the oxytocin molecule. These facts, and a few assumptions, led to a postulated structure (*61*) for oxytocin:



Then, in a preliminary summary (*59*) of the total synthesis of oxytocin and the comparison of the synthetic product with the isolated natural material, satisfactory agreement was reported between the two samples in the following respects:

Biological activity. The synthetic and natural materials were equally effective in inducing labor, and a 1-γ dose of either sample injected intravenously into lactating women produced milk ejection 20 to 30 seconds later.

Countercurrent distribution. The same partition peaks were obtained.

Optical activity. Samples showed the same index of rotation.

Infrared absorption. Samples showed excellent agreement.

Flavianate derivatives. The flavianates agreed in microscopical examination of crystal properties and melting points. The mixed melting point showed no depression.

Hydrolysis and amino acid ratios of synthetic material. Found: leucine, 1.00; isoleucine, 1.00; tyrosine, 0.83; proline 0.92; glutamic acid, 0.91; aspartic acid, 0.93; glycine, 0.98; cystine, 0.87; and ammonia, 3.04; theoretical values are 1.00 for all amino acids listed and 3.00 for ammonia. All amino acids are of the L-configuration.

In recognition of his brilliant study and synthesis (*22, 40, 55, 60*) of oxytocin, as well as his work on the analytical determination of the structure of vasopressin, the pressor hormone of the posterior pituitary, du Vigneaud has received the Nobel Prize in Chemistry.

INSULIN

Last year, after 10 years' work at Cambridge University, Sanger and his colleagues reported the complete structure of insulin as deduced by analytical methods. An article by Thompson (*56*) gives a readable popular account of some of this work and some vivid analogies which illustrate the problems and procedures in determining amino acid sequences in polypeptides or proteins.

Insulin was first crystallized (*1*) in 1926. In 1938 x-ray diffraction showed (*16*) that the crystals had a unit cell which contained three identical or closely similar units each with a molecular eight of about 12,000. For the following decade there was some disagreement as to the molecular weight, until Gutfreund was able to show that on extreme dilution, or in acid solutions, the molecular weight by osmosis approached 12,000 (*19*). About this time it had become apparent that many of the early preparations may have contained zinc, which caused easy crystallization of insulin, but was not a part of the molecule (as it could be dialyzed out without loss of hormone activity).

Sanger collected (*44*) the best analytical data then available on the amino acid contents of insulin (*8, 25, 39, 57*) (Table I).

Table I. Amino Acid Content of Insulin

Amino Acid	Total Protein N, %	Number of Residues, Mol. Wt. 12,000
Arginine	6.35	2
Histidine	8.55	4
Lysine	3.10	2
Glutamic acid	11.4	15
Aspartic acid	4.5	6
Amide N	8.95	12
Cystine/2	9.36	12
Tyrosine	6.5	9
Alanine	4.4, 4.75	7
Valine	6.0, 6.8	8
Phenylalanine	4.4, 4.3	6
Serine	4.45	6
Threonine	1.57	2
Leucine	8.95, 9.2	12
Isoleucine	1.98, 1.83	3
Glycine	5.2, 5.5	7
Proline	2.0, 2.3	3

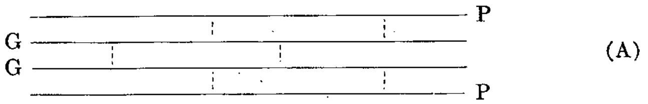
The total of the nitrogen accounted for is 98.5%.

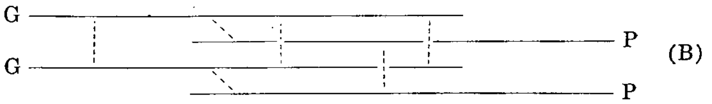
Insulin, treated with 2,4-dinitrofluorobenzene (*45*) took on a total of six *N*-dinitrophenyl groups, two on phenylalanyls, two on glycyls, and two on the ε-amino of lysyls, as shown by hydrolysis and isolation of dinitrophenyl-phenylalanine, dinitrophenylglycine, and dinitrophenyl-lysine.

Sanger and Ryle (*47*) found that a bewildering assortment of cystine-containing peptides could result during hydrolysis of insulin, and studied the conditions necessary to avoid the migration.

Oxidation of the cystinyl —S—S— links with performic acid (*46*) produced two large peptide chains. Chain G had glycine terminal groups, contained no threonine, proline, arginine, histidine, or phenylalanine, and could be separated readily from the second fragment, chain P, by difference in solubility. Chain P was slightly basic, whereas chain G was acidic and soluble in a buffer of pH 6.5. Chain P had a phenylalanine end group and contained all of the remaining amino acids represented in insulin, except for isoleucine.

Sanger considered two over-all structures for insulin:





Sanger preferred A because the thickness (7 to 9 A.) of films obtained in reversible spreading experiments was that corresponding to the thickness of a polypeptide chain. Structure B would require breaking disulfide links under extremely mild, reversible conditions in spreading experiments.

However, the cross linkages to the glycine and phenylalanine chains are not settled, even now. The question will reappear at the end of the discussion of insulin.

Next followed a long and difficult and carefully performed series of experiments by Sanger and Tuppy (*51, 52*) on the amino acid sequence in the P chain from oxidized insulin. Partial hydrolysis with acid (*51*) and proteolytic enzymes (*52*), followed by dinitrophenyl labeling and complete hydrolysis of the peptides to settle the positions of the free primary amino groups in the beginning of each peptide fragment, finally enabled the 30-unit amino acid sequence to be written for the P chain:

1 2 3 4 5 6 7 8 9 10 11 12 13
Phe.Val.Asp.Glu.His.Leu.CySO$_3$H Gly.Ser.His.Leu.Val.Glu.
NH$_2$ NH$_2$

14 15 16 17 18 19 20 21 22 23 24 25 26
Ala.Leu.Tyr.Leu.Val.CySO$_3$H.Gly.Glu.Arg.Gly.Phe.Phe.Tyr.

27 28 29 30
Thr.Pro.Lys.Ala.

The elaborate chart in Thompson's article (*56*) will help the reader to keep track of the manner of deducing sequences from the hydrolytic fragments obtained.

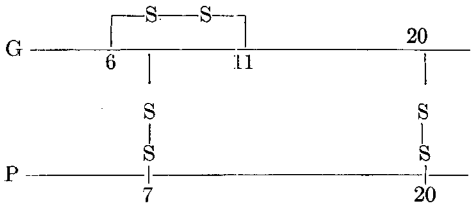
A similar approach with the shorter (21 amino acid residues) but more difficult (fewer key groups occurring only once) sequence in the G chain was completed about the end of 1952 by Sanger and Thompson (*49*). The sequence of the G chain was determined as:

1 2 3 4 5 6 7 8 9 10 11
Gly.Isoleu.Val.Glu.Glu.CySO$_3$H.CySO$_3$H.Ala.Ser.Val.CySO$_3$H.
NH$_2$

12 13 14 15 16 17 18 19 20 21
Ser.Leu.Tyr.Glu.Leu.Glu.Asp.Tyr.CySO$_3$H.Asp.
NH$_2$ NH$_2$ NH$_2$

Indispensable parts of the work on these amino acid sequences were Sanger's labeling with 2,4-dinitrofluorobenzene, Martin and Synge's paper chromatographic methods for separating amino acids and peptides, and preliminary sorting of hydrolysis products into acidic, basic, or neutral groups by electrophoresis or ion exchange separations.

The CySO$_3$H groups in the above sequences marked the positions involving either cross linkages between peptide chains or cyclization within the chains. Sanger, Smith, and Kitai proposed (*48*) the following system of —S—S— linkages for insulin:



The numbers are the group numbers in the G and P chains given above.

The only remaining point to be settled chemically is the number and placement of —$CONH_2$ groups. Glutamic acid and glutamine would appear in acid hydrolyzates as glutamic acid; aspartic acid and asparagine would be aspartic acid. Sanger, Thompson, and Kitai (*51*) treated the G fragment of performic acid-oxidized insulin with mold protease and obtained cleavage of peptide linkages 2—3, 4—5, 11—12, 13—14, 14—15, 15—16, 16—17, 17—18, and to a lesser extent 6—7. Cyanide-inactivated papaine cleaved 4—5, 11—12, 15—16, 16—17, 17—18, and slightly 1—2, 13—14, and 14—15.

Fragment P was cleaved by protease between 1—2, 3—4, 7—8, 10—11, 13—14, 15—16, 16—17, 23—24, 25—26, 28—29, 29—30 and to a lesser extent 11—12, 13—14, and 18—19.

The amide-containing peptides were detected by CO—NH_2 estimations and by ionophoric mobilities. The —$CONH_2$ groups were placed at 5, 15, 18, and 21 of the G chain, and 3 and 4 in the P chain.

The empirical formula for ox insulin derived from the sequences of amino acids is $C_{254}H_{377}N_{65}O_{75}S_6$, molecular weight 5733. This is about half the molecular weight of 11,550 discussed by Arndt and Riley (*3*) in 1953 before the complete sequence was worked out. If, as is suggested by x-ray diffraction and osmotic pressure measurements, the molecular weight is about 11,500 for (ox?) insulin, Sanger's structure must involve —S—S— interchain cross linkages between two units of group weight 5733, in such a way that the film thickness, found in spreading experiments, will also be satisfied.

α-LIPOIC ACID

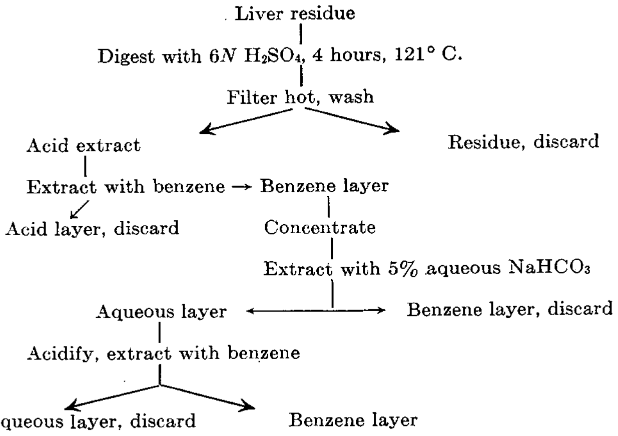
α-Lipoic acid is the acetate-replacing factor in growth-promotion of lactic acid bacteria and is also required in the oxidative decarboxylation of pyruvates. Protogen (*12*) contains this factor and is essential for the growth of the protozoan *Tetrahymena geleii.*

Assays are usually made by a manometric method with cultures of *Streptococcus faecalis* resting cells and are expressed as "pyruvate oxidation factor" (POF) units, where the unit is defined as that given by 1 mg. of Fleischmann Type 3 yeast extract.

There are several natural sources of pyruvate oxidation factor activity, but one of the richest and most easily available is beef liver residue, which contains 1.6 to 3.2 γ per gram. About 10 tons of liver residue were used in development of the isolation method; the eventual recovery was about 2.5% of the activity as 30 mg. of crystalline (+)-α-lipoic acid. The whole process (*38*) is a complicated chore which is summarized in Table II.

The benzene concentrate contained, in addition to the lipoic acids, all the fatty acids released in the original acid digestion. Several methods of purification were tested: countercurrent distribution, conversion to methyl esters, and chromatography of these on alumina or Fluorisil. Two active substances were found, α-lipoic acid and β-lipoic acid. The α to β ratio in the benzene concentrate was about 2 to 1. The more polar β-lipoic acid could be separated without difficulty by distribution methods.

Table II. Isolation of α-Lipoic Acid



POF activity 750 to 1500 units/mg. solutes

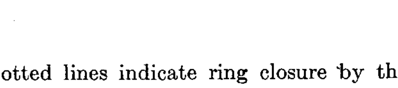
Eventually, some of the methyl ester of α-lipoic acid, assaying 27,000 units per milligram, was isolated from a Fluorisil column as an eluate fraction containing 88% of the original activity at a concentration of 172,000 units per milligram. This was saponified under nitrogen with 0.1*N* potassium hydroxide at 25° C., acidified, and extracted with benzene. The extract was dried in vacuo, dissolved in a minimum of Skellysolve B, cooled, and scratched to induce crystallization.

The pale yellow crystals melted at 47.5° C., and showed $[\alpha]^{25}_{D}$ = 96.7° (in benzene), and a pyruvate oxidation factor activity of about 15,000,000 units per milligram. Ultimate analysis showed a composition corresponding to $C_8H_{14}O_2S_2$, and the neutralization equivalent was 217. The pK'_A was 4.76 for the carboxyl.

Infrared spectrophotometry showed absorptions at 3.37 microns (C—H), 3 to 4 microns (carboxyl O—H), 5.8 microns (COOH), 6.90 microns (CH_2), 8.0 microns (C—OH), and 10.7 microns (COOH dimer). No methyl absorption could be detected. Polarography showed a —S—S— band, the only sulfur function reducible at the dropping mercury electrode (*63*).

α-Lipoic acid was desulfurized with Raney nickel to give *n*-octanoic acid, which was identified by x-ray powder pattern comparison of its silver salt (*26*) with an authentic sample.

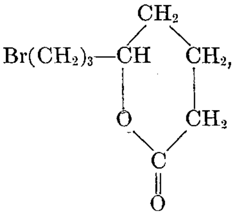
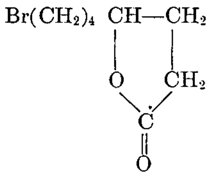
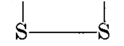
The structural formula is therefore



where the dotted lines indicate ring closure by the —S—S— group from C_8 to C_4, C_5, or C_6, although the 6,8-thioctic acid structure was considered most probable.

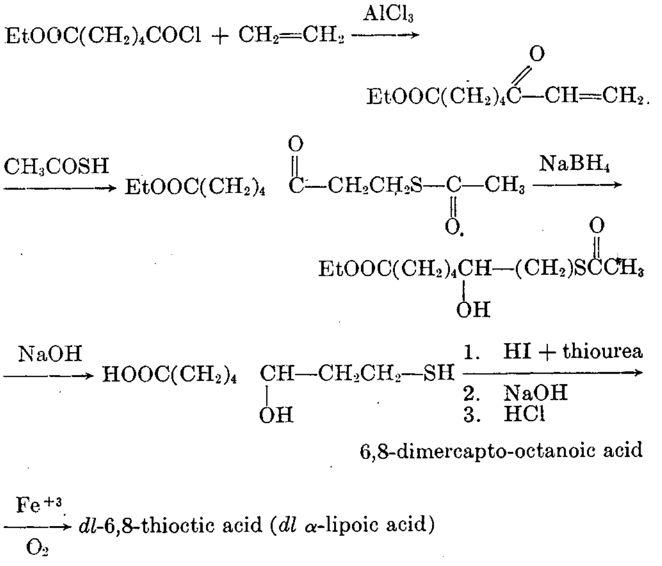
By interconversion of the α- and β-lipoic acids it was established (*37*) that β-lipoic acid is a sulfoxide. The oxidative formation of the β acid from the α acid may have actually occurred during the first step of the isolation procedure.

α-Lipoic acid may react in the dimercaptan form with a number of natural thiols—e.g., methionine and reduced glutathione—to form a variety of disulfides which may differ markedly from α-lipoic acid in benzene solubility. This may provide an explanation for the 2.5% recovery of pyruvate oxidation factor activity from liver residue.

The isolation procedure was not improved at this time because racemic α-lipoic acid was soon available synthetically. The synthesis (21) had drawbacks. The yields were only about 4% from an intermediate,  which can rearrange to  or react in other ways with thiourea and acid to give materials which, after hydrolysis and ring closure, produce  $\underset{S\text{———}}{(CH_2)_x}\text{—}\underset{S}{CH}(CH_2)_yCOOH$, where x and y are not established.

However, the right substance was made, as shown by the exact agreement between the infrared absorption spectra for natural (+)-α-lipoic acid and the synthetic racemic material. The biological activity of the synthetic racemic material was almost half that of the natural (+)-α-lipoic acid, which is what one would expect if the (−)-α-lipoic acid is biologically ineffective.

A little earlier, a group at the Lederle Research Laboratory (12) had reported the isolation of a material called "Protogen A," which appeared to be the same as α-lipoic acid in analysis, melting point, and infrared spectrum. This group improved considerably (32) on the synthesis of the 6,8-thioctic acid:



This approach had the advantage of better yields (30% overall from the thiolacetate to the final product) and certainty as to the ring size in the product, which melted at 58° C.

Finally, a group at Merck (62) reported the preparation of (+)-α-lipoic acid, $[\alpha]^{23}_{D}$ + 104° (in benzene), melting point 46–48° C. The (−)-α-lipoic acid, melting point 45–47.5° C., $[\alpha]^{23}_{D}$ − 113° (in benzene), was biologically almost inactive. A mixture of equal parts of (+) and (−)-lipoic gave the racemate, melting point 60–61° C.

VITAMIN B_{12}

Since 1926 (27) it has been recognized that there is in liver some substance which is essential for the development of red blood cells and hemoglobin in human bone marrow. Lack of this factor causes pernicious anemia.

Up to 1947 or 1948 the isolation and identification of this factor was hindered by the scarcity of patients with pernicious anemia, for use in assays. In 1948 Shorb reported (53) the use of *Lactobacillus lactis* Dorner (LLD), for which growth depended on liver extracts and particularly upon traces of a red, crystalline substance (41) isolated from liver extracts by a group of chemists at Merck & Co., Inc. The red crystals melted at over 300° C., darkened rapidly at 210° to 220°, showed refractive indices of α 1.616, β 1652, and γ 1.664, and contained cobalt and phosphorus. The crystals also caused quick alleviation of symptoms in patients with Addisonian pernicious anemia, even in doses of 3 to 6 γ.

In 1948 a similar substance was isolated in England from cultures of *Streptococcus griseus*. This material showed the same melting behavior, the same indices, contained cobalt and phosphorus, and showed an LLD response of 11.7×10^6 units per milligram, as compared to 11.0×10^6 units per milligram for the Merck sample (42).

In 1949 the Merck group found 5,6-dimethylbenzimidazole as a hydrolysis fragment of the vitamin.

In 1953 the English chemists reported (2) that under acidic or basic conditions vitamin B_{12} is cleaved to give 1-amino-2-propanol, five equivalents of ammonia, 5,6-dimethylbenzimidazole, 1-α-D-ribofuranoside-2′(or 3′)-phosphate, and a red cobalt-containing gum which contained $R(COOH)_{4,5,\text{ or }6}$ from alkaline hydrolysis, or $R(COOH)_{4,5,6,\text{ or }7}$ from acid hydrolysis.

Cannon, Johnson, and Todd reported (13) later that the red gum from alkaline hydrolysis was separated into several fractions by paper electrophoresis. The major component $R(COOH)_6$ was converted to the ammonium salt, ion exchanged on a column of Dowex 1-X-2, and eluted by dilute ammonium acetate followed by several minor impurities. $R(COOH)_6$ was then extracted with phenol, washed with water to remove inorganic impurities, diluted with ethyl ether, and extracted with water. The water layer was evaporated to dryness and the residue was dissolved in a small volume of acetone. After several weeks, the acetone solution had deposited dark red prismatic crystals of the composition $C_{47}H_{66}O_{16}N_6CoCl$. The ultraviolet spectrum of a solution of the crystals showed a considerable resemblance to the spectrum of vitamin B_{12}, with differences due to the absence of the dimethylbenzimidazole nucleotide in the hydrolysis product.

There was a bathochromic shift in the presence of cyanide ion. The infrared spectrum of the cyano complex showed CN and —COOH groups and no aromatic absorptions.

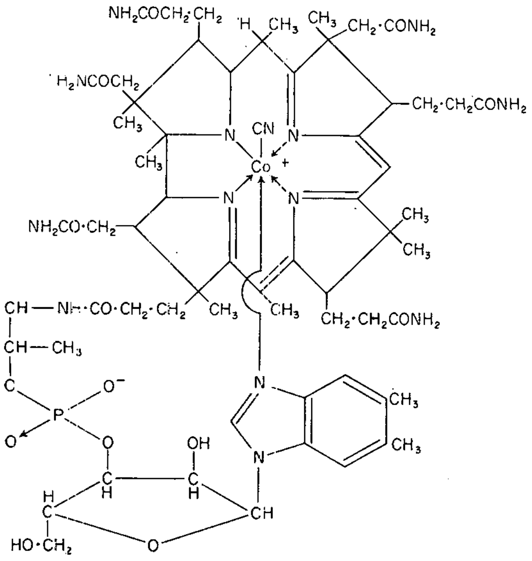
The multiplicity of eluates from electrophoretically pure material in solutions of various ions (acetate, etc., but not cyanide) is probably due to anion exchange.

The Oxford group then made detailed x-ray crystallographic studies (9) of vitamin B_{12} and the $R(COOH)_6$ fragment isolated as described above, substituting selenocyanate for cyanide in the acid fragment. Certain features of the two substances were similar.

Since the 1954 x-ray work, crystallographic studies have continued at Oxford, Princeton, and University of California at Los Angeles. The tedious chore of many calculations necessary for the structure factors and three-dimensional Fourier syntheses has been shortened by the use of the National Bureau of Standards automatic computer at Los Angeles (20).

The Merck group has recently isolated two fragments from the chromic acid oxidation of vitamin B_{12} hydrolyzates (23).

While the structure of the degradation product is now fairly well settled, the structure of the vitamin itself is incomplete regarding the arrangement of double bonds. A provisional structure for vitamin B_{12} and a summary of the chemical evidence for it have been given by the Cambridge group (*7*).



This problem of the structure of vitamin B_{12} is eloquent testimony to the results that can be obtained quickly by chemists and x-ray crystallographers working together. A similar case was that of penicillin during the war (*17, 43*).

ACKNOWLEDGMENT

The reviewer gratefully acknowledges his indebtedness to Alice V. Neil, librarian at the General Electric Co. Research Laboratory, for help in securing certain references.

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