ANALYTICAL CHEMISTRY

NOVEMBER 1956

Lisbon Congress—An Outstanding Success

LISBOA! How can anyone who attended the recent International Congress on Analytical Chemistry forget the cordial, indeed, warm hand of hospitality of the people of Lisbon—not just those in charge of the congress but every citizen of that beautiful city. The streetcar conductor, the taxi driver, the merchant, the person on the street, all were imbued with a desire to extend the hand of welcome to those who had come to their city as international guests.

While the attractive posters describing the meeting, which were widely displayed on the principal streets, were responsible in part for interest of Lisbon citizens in the meeting, we do not believe this was the principal factor. Lisbon citizens are a gracious and loving type of people, who instinctively extend the hand of friendship while still retaining a proper reserve. The exhibition of proper balance was very instructive to observe.

Elsewhere we have reported on the meeting. Certainly it was a highlight in the history of the progress of analytical chemistry. More than 1400 registered from some 50 countries. As we witnessed this throng the thought ran through our mind,—would such a gathering have been possible even a decade ago? We doubt it.

One of the delightful sidelights of the meeting were some of the English expressions coined for English-speaking participants. We were intrigued immensely, for example, with the term "congressists." Perhaps we have missed this term in the past, but certainly it is definitive and descriptive.

Of specific interest to our readers are publication plans for the papers. The main and the section conferences will be published by Editorial Birkhaüser in Bâle. We understand these lectures will be available very shortly and hope we can provide more definite information regarding their availability in an early issue.

We were advised during our stay in Lisbon that authors of communications (perhaps we would refer to them as papers) are at liberty to offer them to any journal of their choice. There is still the possibility they will be included in a single volume, but our Portuguese conferees recognized the desires of many authors for prompt publication.

We cannot close without seconding the views of those in official circles, that future analytical conferences be held as part of the general conferences and congresses of the International Union of Pure and Applied Chemistry.

Perhaps we are somewhat biased in our strong endorsement of this principle. The science of analytical chemistry now cuts across the broad fields of biology, chemistry, physics—indeed, can one name any scientific discipline not now dependent upon progress in what we loosely define as analytical chemistry? This in itself is to us a most convincing reason why future international analytical congresses should be associated in time and place with IUPAC conferences and congresses.

Walter J. Murphy

Separation of Porphyrins by Paper Chromatography

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A paper chromatographic method has been developed for the resolution of the aggregate of porphyrin pigments occurring in ancient sediments and petroleum. In a two-dimensional chromatogram the nonacidic pigments, the esters, and the metal complexes of both are separated first. The free acids which remain at the origin are then esterified with diazomethane. A second development at a right angle to the first one resolves the newly formed methyl esters of the acidic pigments and their metal complexes. The location of the spots on the completed chromatogram can be correlated with structural features of the porphyrins. As little as 5×10^{-9} gram of free porphyrin and 4×10^{-8} gram of the nonfluorescent metal complexes can be detected; a rapid semiquantitative determination is possible by comparison with a series of standards or by measurement of the spot areas.

ALL porphyrin pigments can formally be deduced from one parent compound, the porphin. A very large number of substituted porphins are known today; most of them have been prepared synthetically, and a considerable number have been isolated from biological sources. Very few of these porphyrins have been found in petroleum and ancient sediments. These fossil pigments apparently are the most stable end members formed from the chlorophylls and hemins through a series of transformation reactions. Most abundant in petroleum and ancient sediments are the two chlorophyll derivatives, deoxophyllerythrin (DP) and deoxophyllerythroetioporphyrin (DPEP) [for the structural formulas see Fischer and Orth (*7*)]. In coals, two other pigments, mesoporphyrin (MP) and mesoetioporphyrin (MEP, also designated as etioporphyrin III) are more abundant.

The porphyrins occur in sediments and petroleum, both free and as complexes with heavy metal ions. The most abundant pigments are the vanadium complexes of deoxophyllerythroetioporphyrin and deoxophyllerythrin (DPEP-V and DP-V). Two other types of complexes have been described as containing nickel (*8*) and iron (*10*) as the central atoms. Complexes of other metals have never been identified with certainty.

The presence of porphyrins in geological materials was described for the first time in 1934 by Treibs (*13*). Historically, this discovery is of great importance because it established for the first time a direct link between constituents of crude oil and their parent compounds in the biological source material. With the finding of porphyrin metal complexes, Treibs also indicated that organic molecules can be the carriers and possibly also the concentrators of heavy metals in petroleum and oil shales.

The occurrence of these pigments in petroleum and ancient sediments is still of great interest; it was therefore decided to review the available methods for the determination of porphyrins in geological materials.

DETERMINATION OF PORPHYRINS IN GEOLOGICAL MATERIALS

The porphyrin pigments possess a typical absorption spectrum with strong bands in both the visible and near-ultraviolet regions. The spectra are sensitive to differences in the type of substitution on the porphyrin ring and can be used for at least a tentative structural analysis of the pigments. Unfortunately, the spectra of many porphyrins with similar structure are almost identical. Mesoporphyrin and mesoetioporphyrin, for instance, and also deoxophyllerythrin and deoxophyllerythroetioporphyrin cannot be distinguished by spectroscopy in the ultraviolet and visible regions. The spectra of the metal complexes are determined by the complexing metal ion rather than by the substitution of the porphin ring. Absorption-spectroscopic methods (*9*), although they present a very sensitive tool for the detection of porphyrins, cannot provide the necessary detailed information about the relative contribution of individual closely related pigments in a fossil porphyrin mixture. This information can be obtained only by a separation of the mixture by some selective method and a consecutive determination of the individual pigments.

Methods for the isolation of individual porphyrins from mixtures have been described by Treibs (*14*). These separations, based on fractionated chromatography and stepwise solvent extraction, are extremely tedious and cannot be carried out as routine methods. Improved techniques for the isolation of porphyrins from biological materials have been developed recently. With paper chromatography, they effect an excellent and fast resolution of the pigments and require only very small samples.

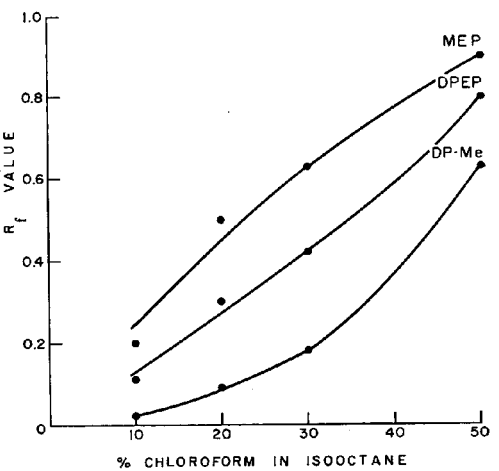

Figure 1. R_f values of porphyrins in chloroform–iso-octane mixtures

Whatman No. 3 paper

According to Nicholas and Rimington (*11*), free porphyrin carboxylic acids can be separated by a development with a lutidine-water mixture in an ammonia atmosphere. An inverse linear relationship exists between the R_f values of the porphyrins and the number of carboxyl groups in the molecule. This method in its original form or in modifications by Corwin and Orten (*5*) or Eriksen (*6*) has found wide application in studies of the metabolic products of porphyrins.

Another method, described by Chu, Green, and Chu (*4*), permits the separation of the porphyrin methyl esters. A chloroform-kerosine mixture first serves as developer. After the chromatogram has been dried, a second development follows in the same direction, this time with a 1-propanol–kerosine mixture. This unidirectional double development appeared necessary for a satisfactory resolution between the adjacent spots and for reduction of trailing. Here again, an inverse relationship is found between the R_f values and the number of the (esterified) carboxyl groups on the porphyrin ring. The method has been modified by Bogorad and Granick (*3*), who use the same developer systems but apply them in a two-directional double development. Their technique permits a better resolution of adjacent spots, especially if they are caused by the presence of very different quantities of pigments.

Another modification has recently been described by Rappoport and associates (*12*), who use the high degree of resolution obtained by a circular development. The method requires relatively large samples, however, and necessitates elution of the pigments for quantitative determination.

All these methods show a serious lack of resolution for the etioporphyrins and porphyrin mono- and dicarboxylic acids which are most abundant in geological materials. Attempts were made, therefore, to find developer systems that would spread these pigments more evenly over the paper. The ability to differentiate sharply between the free acid group and the ester-etioporphyrin group in Chu's technique is very desirable. Several difficulties, however, prohibit the application of this method to the separation of fossil porphyrins. In the first place, mesoetioporphyrin and deoxophyllerythroetioporphyrin are not resolved, because both migrate with the solvent front. The one-dimensional double development is time-consuming and requires close attention during the development. Kerosine is not a desirable component of a developer because of its nonreproducible composition and low volatility. During the slow drying of the chromatograms, an increase of the spot areas has been observed. This prohibits the direct quantitative estimation of the porphyrins by scanning for light absorption or by measurement of the spot areas.

It was hoped that most of the difficulties mentioned above might be overcome by a better choice of solvents and paper. Preliminary experiments indicated that a single development did not necessarily lead to heavy trailing, if the paper was properly selected. Contrary to Chu's report, no loss of sensitivity in the porphyrin detection was found, if a pure hydrocarbon like iso-octane was substituted for the kerosine as the paraffin component of the developer system.

EXPERIMENTAL

The reagents used were iso-octane (2,2,4-trimethylpentane), Phillips Petroleum Co., pure grade; carbon tetrachloride, Mallinckrodt, analytical reagent grade; and fluoranthene, Matheson, Coleman and Bell.

Whatman No. 3 filter paper is recommended for general use because of the reduced tendency for trailing.

The chromatograms were carried out on the filter paper supported in a horizontal position in a large desiccator, the walls of which were lined with heavy filter paper wetted with solvent in order to keep the atmosphere in the developing tank saturated. Any other development technique (ascending, descending) might be used. The chromatograms are developed in the direction of the grain of the paper; in the two-dimensional separations, the first separation is carried out in this direction. Standard chromatographic techniques were used for spotting, developing, and drying of the sheets and the planimetry of the spots.

Esterification of Spots by Diazomethane. The spot of the acidic porphyrins which remains at the origin after the first development can be esterified with either diazomethane gas or a solution. The reaction of the methylating agent has to be restricted to the immediate area of the spot because excessive methylation of the paper would change the R_f values.

In practice, this is easily achieved by slowly adding a solution of diazomethane from a capillary pipet to the spot, balancing the rate of addition and the rate of evaporation of the solvent. Cyclohexane has been selected as a solvent that dissolves a sufficient amount of diazomethane but will not affect the porphyrin spots. Diazomethane is prepared from either nitrosomethylurea or *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (*2*) (Eastman organic chemicals). Nitrosomethylurea is not available commercially, but small quantities as needed for this procedure are rapidly prepared following Weygand's procedure 1 (*15*, p. 261). Nitrosomethylurea is unstable at room temperature and should be kept under refrigeration; because of its toxicity, diazomethane and its solutions should be handled with due care.

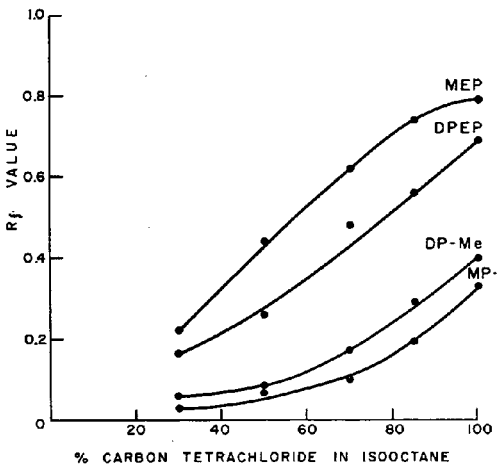

Figure 2. R_f values of porphyrins in carbon tetrachloride–iso-octane mixtures

Whatman No. 1 paper. R_f values on this paper are very close to those obtained on No. 3 paper.

Diazomethane in Cyclohexane. In a well-ventilated hood, 10 ml. of cyclohexane and 10 ml. of 20% aqueous potassium hydroxide are warmed to about 35° C. in a 50-ml. Erlenmeyer flask. One hundred milligrams of nitrosomethylurea is dropped into the hydroxide layer. As soon as all of it has reacted, the flask is cooled in ice. The diazomethane solution is withdrawn for use with a capillary pipet.

Porphyrins. The porphyrins used for the separations described here were prepared in this laboratory. Hemin (Eastman organic chemicals) served as a starting material for the preparation of protoporphyrin (PP) (removal of iron by treatment with iron powder and formic acid), of mesoporphyrin (catalytic hydrogenation of protoporphyrin), and of mesoetioporphyrin (decarboxylation of mesoporphyrin). The vanadium complexes of deoxophyllerythrin and its etioporphyrin were isolated from a porphyrin-rich Triassic oil shale from Serpiano, Switzerland (*1*). Free deoxophyllerythrin and deoxophyllerythroetioporphyrin were prepared from the vanadium complexes and the nickel and copper complexes were synthesized from nickel or copper acetate and a solution of the porphyrins in acetic acid. The methyl esters of the porphyrins were prepared by esterification of the pigments with diazomethane.

Selection of Solvent Systems. As a consequence of the preliminary experiments, a series of chromatograms was prepared in which chloroform–iso-octane mixtures of varying compositions were used as developers (Figures 1 and 2).

No trailing was observed, and a good resolution between mesoetioporphyrin and deoxophyllerythroetioporphyrin was obtained, with a maximum at about 30% chloroform in the developer mixture.

Replacement of the polar component, chloroform, by the unpolar solvent, carbon tetrachloride, should decrease the R_f values

of the porphyrins in a developer with comparable concentration of chlorinated hydrocarbons. This effect was confirmed experimentally (Figure 3). Again, an excellent resolution of mesoetioporphyrin from deoxophyllerythroetioporphyrin and DP-Me (Me is used to characterize a methyl group) from MP-Me_2 took place.

The relative merits of the two different developer types were compared in a series of chromatograms using two developers giving an equal R_f value for one standard porphyrin (DP-Me) and giving also the highest degree of resolution possible within the chloroform and carbon tetrachloride developer series. The R_f values for several porphyrins were then determined in the two systems, and the resolution was calculated as the difference in the R_f values (Table I).

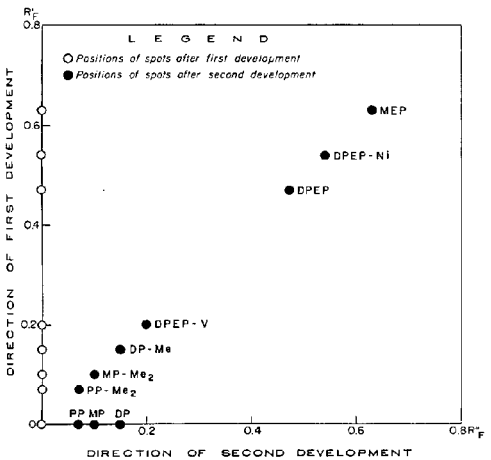

Figure 3. Two-dimensional separation of porphyrins

The resolving power of the carbon tetrachloride system is better than that of the chloroform system. Carbon tetrachloride–iso-octane mixtures are therefore recommended for general use. The relative concentration of the two components of the developer can be adjusted to conform to the type of porphyrin mixture to be separated. If only etioporphyrins are present, a low R_f developer containing 50 to 70% carbon tetrachloride is used; if esters have to be resolved, a developer containing between 70 and 85% of the same solvent is preferred.

Selection of Filter Papers. The choice of the most suitable type of filter paper is one of the many factors which influence the success of a paper chromatographic separation. On its texture and composition depend, among other factors, the rate of migration of the mobile phase, the degree of resolution, and the amount of trailing of the spots. In order to find the best conditions for the separation of porphyrins, chromatograms were carried out using Whatman filter papers 1, 2, 3, and 4. The R_f values were determined and the shape of the spots and the amount of trailing were compared. The R_f values obtained on the four papers are identical within ±0.05 unit, but a great difference exists in the appearance of the spots. Minimum trailing, maximum resolution between adjacent spots, and the sharpest definition of the spots are obtained on the thick filter paper, Whatman No. 3. The quality of the separation decreases from this paper to Whatman No. 1 and is insufficient on No. 2 and No. 4. The technique of unidirectional double development had been devised by Chu, Green, and Chu in order to overcome the serious amount of trailing found on Whatman No. 1 filter paper. With the Whatman No. 3 paper, a single development is satisfactory. Elimination of the second development results in a considerable saving of time as compared to the original method.

Separation of Acidic from Nonacidic Porphyrins by Two-Dimensional Development. When mixtures of porphyrin carboxylic acids with etioporphyrins and esters are chromatographed, an unresolved spot representing the acids remains at the origin. A simple method for their subsequent resolution has been found.

A square sheet of filter paper is spotted with the initial mixture in one corner and then developed as described above. The solvents are removed by evaporation, and the unresolved spot at the origin is then made to react with diazomethane. After a few seconds, the acidic porphyrins are completely esterified. The paper is then developed a second time with the same solvent but at a right angle to the first development. The pigments that migrated during the first development migrate again with the same R_f value and reach a position on a diagonal line over the paper. The esters that were formed by the reaction with the diazomethane now migrate away from the origin and are found along one edge of the sheet (Figure 4).

Relationship between R_f Value and Constitution of Porphyrins. In normal phase chromatography, the solutes are distributed between the more polar stationary phase and the less polar mobile one. The distribution coefficients are influenced by the constitution of the solutes. Relatively small differences in the polarity of similar compounds can cause a change of the partition coefficient and R_f value of sufficient magnitude to permit a separation. Systematic changes of the R_f values are frequently found between the members of homologous series of compounds and can be used in the determination of the structure of unknowns.

Table I. Resolution of Porphyrins by Different Developer Solvents

Development. Horizontal in saturated atmosphere of the solvent vapor
Paper. Whatman No. 3
Developers. Iso-octane containing 30 volume % chloroform, and iso-octane containing 70 volume % carbon tetrachloride

Porphyrins	Difference in R_f Value in System: Chloroform	Carbon tetrachloride
MEP + MP-Me_2	0.42	0.52
DPEP + DP-Me	0.22	0.32
DP-Me + PP-Me_2	0.05	0.08
DP-Me + MP-Me_2	0.02	0.06
DPEP + MEP	0.18	0.16

The present method for paper chromatography of porphyrins permits a direct correlation of the R_f values with structural properties of the pigments (Figure 5 and Table II). The high R_f values of nonacidic porphyrins—e.g., DPEP and MEP—are reduced to zero upon the introduction of one or more carboxyl groups. Upon esterification, the R_f values increase again, but remain considerably lower than those of the etio pigments. In the series of the esters, the R_f values decrease from the mono- to the dicarboxylic ester (from DP-Me to MP-Me_2 and PP-Me_2). Esters of polycarboxylic acids would have a very low R_f value or would not migrate at all, but they could be resolved with a more polar eluent—e.g., a chloroform–iso-octane mixture containing a high percentage of chloroform.

Upon complexation with a metal like copper or nickel, the porphyrin loses two strongly polar sites (the —NH groups of the pyrrole rings), and the R_f values increase slightly. Complexation with vanadium introduces the oxygen atoms of the vanadyl ion, increasing the polarity of the molecule and lowering the R_f value.

Even very small differences in the substitution of the porphin system show up clearly. DPEP with the isocyclic five-membered ring is well separated from MEP with an open side chain and the same number of carbon atoms. MP-Me_2, which does not have any double bonds in the side chains, moves slightly faster than PP-Me_2 with two vinyl groups, and can be separated from it on a sufficiently large sheet of paper.

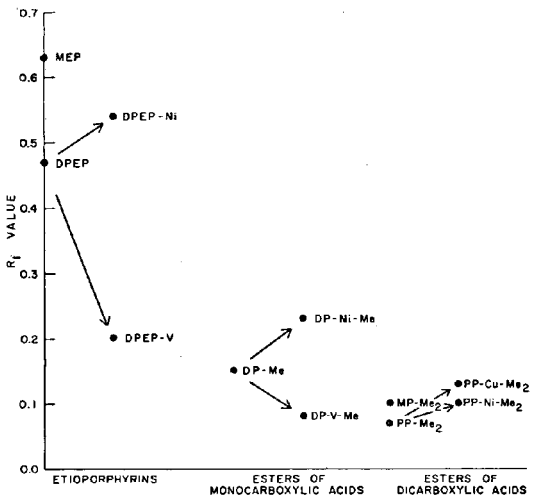

Figure 4. R_f values and constitution of porphyrins

Limit of Detection and Usable Range of Method. After the separation, the porphyrin spots can be very easily located on the paper by observing their fluorescence in long-wave ultraviolet radiation. Wetting the sheet with iso-octane during the observation helps in obtaining a higher sensitivity and better visual resolution of adjacent spots. Impregnation with iso-octane makes the paper translucent and increases the amount of fluorescent radiation that reaches the eye by decreasing scattering and absorption. Iso-octane does not dissolve the porphyrins and therefore leaves the spots unaltered for a subsequent quantitative determination. A very low limit of detection can be realized in this manner; for mesoporphyrin dimethyl ester it was found at 0.005 γ. This limit is distinctly lower than the one reported by Chu, Green, and Chu, in disagreement with their statement that the use of pure alkanes in place of kerosine reduces the fluorescent intensity of the porphyrin spots.

Phorphyrin metal complexes lack the sensitive fluorescence of the uncomplexed pigments, and their characteristic color can be detected only at relatively high levels of concentration, where the danger of trailing exists. An indirect method for their detection, making use of their intense ultraviolet light absorption, has been developed. For this purpose, the completed and dried chromatogram is briefly sprayed with or dipped in a saturated solution of fluoranthene in *n*-pentane. It is then rapidly air-dried and observed over a source of long-wave ultraviolet (366 mμ). The sheet exhibits an even bright fluorescence except for dark spots where porphyrin-metal complexes or free porphyrins absorb the ultraviolet radiation and prohibit the fluorescence of the fluoranthene.

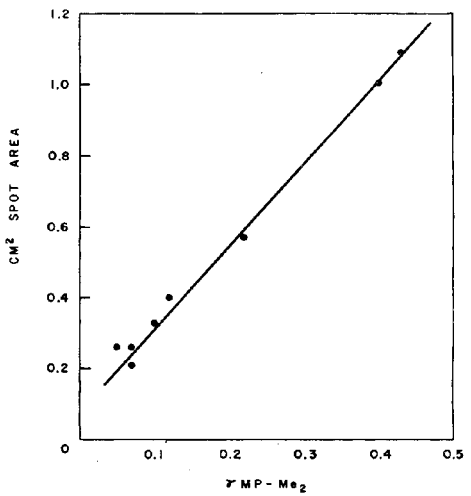

Figure 5. Calibration curve for determination of mesoporphyrin dimethyl ester from spot area

With this technique, very small quantities of porphyrin metal complexes can be detected; for DPEP-V the limit of detection lies at 0.04 γ. Compounds that have a strong ultraviolet absorption and are not separated from the porphyrins during the development will interfere with this method of detecting the metal complexes.

The usable range of a paper chromatographic method is limited by two concentrations, beyond which separation and accurate determination are impossible. The lower one of these values is identical with the limit of detection. The upper limit is determined by the maximum capacity of the mobile and immobile phases for the compounds. At higher concentration, the initial solid or liquid phase is dissolved only gradually upon development causing the formation of trails. The limit at which this trailing appears depends on the initial spot area, the thickness of the paper, the solubility of the solute in the developers, and other factors that affect the capacity of the mobile and immobile phases. Under the experimental conditions described above, trailing of a porphyrin takes place when its concentration in the porphyrin mixture is equal to or higher than 0.5 γ.

The two limits discussed above restrict the useful range of the method to about 0.5 to 0.005 γ for the free porphyrins and 0.5 to 0.04 γ for the metal complexes.

Table II. R_f Values of Free Porphyrins, Their Metal Complexes, and Methyl Esters

Development. Horizontal in saturated atmosphere of solvent vapor
Paper. Whatman No. 3
Developer. Carbon tetrachloride containing 30 volume % iso-octane

Porphyrin	R_f Value
MEP	0.63
DPEP-Ni	0.54
DPEP	0.47
DPEP-V	0.20
DP-Ni-Me	0.23
DP-Me	0.15
DP-V-Me	0.08
MP-Ni-Me_2	0.12
MP-Me_2	0.10
PP-Cu-Me_2	0.13
PP-Ni-Me_2	0.10
PP-Me_2	0.07

Table III. Estimation of Porphyrins by Comparison with Standards

Development. Horizontal in saturated atmosphere of developer vapor
Paper. Whatman No. 3
Developer. Carbon tetrachloride containing 30 volume % iso-octane
Porphyrin. Mesoporphyrin dimethyl ester

Porphyrin Present, $\times 10^{-6}$ G.	Porphyrin Estimated, $\times 10^{-6}$ G.	Deviation from True Value, %
0.04	0.05	+20
0.07	0.06	−14
0.10	0.08	−20
0.40	0.3	−25

SEMIQUANTITATIVE DETERMINATION OF PORPHYRINS

From the point of view of speed and simplicity, methods for the quantitative determination of chromatographically separated compounds can be grouped in two classes. Into one class fall methods for a direct evaluation of the spots on the filter paper (comparison with a series of standards, measurement of spot area or diameter, scanning for light absorbance, fluorescence, or radioactivity). Into the other class fall methods that necessitate the recovery of the separated components from the paper before the determination. Methods in the first group are usually very rapid, but often not so accurate as those in the second group.

Estimation of Porphyrins by Comparison with Standards or Measurement of Spot Area. By far the most simple, but not a very accurate, method of estimating chromatographically separated compounds consists in visual comparison of the spots with a series of standards. Results falling within the limit of ±30% error can be obtained by such an estimation (see Table III). The main advantage of this method is the speed and ease with which the estimation is carried out and the independence of the error from concentration. Most other direct or indirect determinations lose their accuracy in the neighborhood of the limit of detection.

Higher accuracy, especially at higher levels of concentration, can be obtained if the area of the spots is measured and compared with the area of standard spots. Some personal judgment is involved in marking the edges of the spots; calibration and measurement should therefore be carried out by the same person. The area of the initial spots should be kept as small and constant as possible by forced evaporation of the solution during spotting. Large deviations in the initial spot area are reflected in variations of the final spot size. For best accuracy, calibration spots should be carried along on the same sheet that is used for an actual determination.

If all these points are carefully controlled, the error of the determination can be kept within the limit of ±10%. A typical calibration curve for the determination of MP-Me_2 is plotted in Figure 5.

Certain polar compounds which might contaminate a porphyrin concentrated from natural sources have an eluting effect on the porphyrins. The resulting increase in the R_f value causes an increase in the spot area, which will interfere with a quantitative determination by the above method. To avoid this type of interference, a porphyrin determination from the spot area should not be attempted if the R_f value is increased more than 0.03 R_f unit over the value of a pure standard.

The two techniques described are of sufficient accuracy for many purposes; for a more accurate determination, the use of a scanning technique for light absorption or fluorescence or the elution of the porphyrins and a consecutive microspectrophotometric determination of the pigments should be attempted.

CONCLUSIONS

A method developed for the separation of porphyrins that occur in petroleum and sedimentary rocks increases the resolving power from earlier paper chromatographic methods to the point where metal complexes can be separated from each other and from the corresponding free porphyrins. The technical difficulties encountered by Chu, Green, and Chu in the separation of porphyrin methyl esters have been overcome by a careful selection of filter papers and developer solvents, and the need for a one-directional double development has been eliminated. Two-directional development results in a sharp separation of non-acidic and acidic porphyrins and permits the separation of both groups into individual components. The limits of detection of the uncomplexed pigments are lower than previously reported and a sensitive method has been developed for detecting the porphyrin metal complexes which lack the fluorescence of the uncomplexed pigments.

A semiquantitative determination of the pigments is possible by comparison with a series of standards; for better results, measurement of the spot areas is recommended.

The R_f values of the pigments can be used for their identification and also for structural evaluation of porphyrins with unknown structure. They are used in much the same way as the pH and acid values of Fischer and are applicable also to metal complexes and acid-sensitive pigments.

ACKNOWLEDGMENT

The author wishes to express his appreciation to the Shell Development Co. for permission to publish this article.

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RECEIVED for review March 23, 1956. Accepted July 2, 1956. Publication 85, Shell Development Co.

Identification of Silver Salts of Branched-Chain Fatty Acids by X-Ray Diffraction Powder Patterns

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The increasing use of synthetic branched-chain fatty acid mixtures in the preparation of metallic soaps makes the identification of the individual acids important. X-ray diffraction data are presented for 13 silver salt derivatives of hexanoic acid and one derivative of heptanoic acid. In a given isomeric series the long spacing increases as the group is moved further from the carboxylate group.

POWDER diffraction patterns for the anilides of normal saturated fatty acids (C_1 to C_{18}) have been shown by Matthews and Michell (*2*) to be sufficiently characteristic to serve as a means of identification. Matthews, Warren, and Michell (*3*) later presented data on the anilides of three more fatty acids and also x-ray diffraction data on the amides and silver salts of a number of fatty acids. These authors concluded that the silver salts were the most satisfactory derivatives, based largely on ease of preparation and analysis as well as pattern differentiation.

Although data are available for straight-chain fatty acids and some branched-chain acids, little data have been published on a fairly complete series of isomers. The Bureau of Mines (*4*) recently completed a program in which a large number of branched-chain hexanoic acids have been prepared. Some of these acids are components of synthetic fatty acid mixtures currently

Table I. Powder Diffraction Data of Silver Salts of Branched-Chain Fatty Acids

1. 2-Methylhexanoic

d, A.	I/I_1
12.8	1.00
9.74	0.40
8.37	0.30
7.62	0.05
6.17	0.10
5.27	0.05
4.58	0.05
4.49	0.05
4.19	0.10
4.02	0.05
3.92	0.05
3.39	0.05
3.31	0.05
3.23	0.05
3.13	0.10
3.01	0.10
2.68	0.10
2.62	0.05
2.54	0.05
2.46	0.05
2.40	0.05

2. 3-Methylhexanoic

d, A.	I/I_1
15.5	1.00
12.1	0.50
9.77	0.05
8.50	0.05
7.72	0.60
6.70	0.05
6.08	0.10
5.64	0.05
5.13	0.40
4.84	0.05
4.37	0.10
4.19	0.10
4.04	0.05
3.82	0.30
3.57	0.30
3.31	0.05
3.14	0.05
3.05	0.30
2.87	0.05
2.84	0.05
2.78	0.05
2.70	0.05
2.59	0.10
2.49	0.10
2.42	0.05
2.31	0.05
2.26	0.10
2.18	0.10
2.03	0.10
1.96	0.05
1.91	0.05
1.84	0.05
1.70	0.05
1.67	0.05
1.62	0.05
1.52	0.05
1.48	0.05

3. 4-Methylhexanoic

d, A.	I/I_1
15.6	1.00
12.3	0.50
7.78	0.70
6.15	0.20
5.73	0.10
5.16	0.60
4.84	0.20
4.36	0.40
4.10	0.20
3.86	0.50
3.71	0.50
3.17	0.40
3.09	0.10
3.02	0.10
2.93	0.10
2.79	0.05
2.70	0.25
2.60	0.25
2.51	0.25
2.42	0.30
2.35	0.20
2.28	0.15
2.21	0.15
2.18	0.30
2.15	0.05
2.09	0.10
2.06	0.05
2.01	0.10
1.94	0.05
1.87	0.15
1.79	0.15
1.72	0.10
1.69	0.10

4. 5-Methylhexanoic

d, A.	I/I_1
15.8	1.00
13.5	0.60
12.3	0.80
9.44	0.20
8.64	0.20
7.78	0.95
6.70	0.10
6.15	0.60
5.75	0.40
5.20	0.95
4.72	0.05
4.51	0.05
4.33	0.10
4.17	0.10
4.07	0.20
3.87	0.30
3.74	0.20
3.56	0.40
3.17	0.20
3.11	0.30
3.00	0.20
2.97	0.05
2.77	0.05
2.72	0.20

(*Contd.*)

4. 5-Methylhexanoic

d, A.	I/I_1
2.58	0.10
2.47	0.10
2.43	0.05
2.40	0.05
2.36	0.10
2.23	0.20
2.17	0.05
2.09	0.10
2.00	0.20
1.97	0.05
1.93	0.05
1.87	0.10
1.78	0.10
1.72	0.10
1.68	0.05
1.66	0.10
1.60	0.05
1.55	0.05
1.51	0.10
1.49	0.05
1.38	0.05
1.30	0.05

5. 3,5-Dimethylhexanoic

d, A.	I/I_1
16.5	1.00
12.7	0.55
10.9	0.30
8.13	0.55
7.52	0.05
6.47	0.10
5.37	0.50
4.90	0.30
4.52	0.30
4.28	0.30
4.00	0.20
3.90	0.20
3.63	0.40
3.52	0.05
3.43	0.10
3.22	0.30
3.10	0.20
2.93	0.05
2.89	0.05
2.82	0.05
2.70	0.40
2.65	0.10
2.58	0.10
2.52	0.10
2.45	0.10
2.38	0.10
2.31	0.05
2.20	0.20
2.14	0.10
2.10	0.10
2.06	0.10
2.01	0.10
1.94	0.10
1.87	0.05
1.75	0.10

(*Contd.*)

5. 3,5-Dimethylhexanoic

d, A.	I/I_1
1.73	0.10
1.69	0.10
1.64	0.05
1.57	0.05
1.52	0.05
1.47	0.05
1.42	0.05
1.35	0.05

6. 2-Ethylhexanoic

d, A.	I/I_1
13.6	1.00
11.2	0.40
8.43	0.30
6.74	0.35
5.56	0.20
2.72	0.05

7. 3-Ethylhexanoic

d, A.	I/I_1
16.7	1.00
13.3	0.30
11.2	0.40
10.1	0.30
8.35	0.40
7.36	0.10
6.62	0.05
6.04	0.10
5.56	0.10
5.40	0.05
4.78	0.05
4.27	0.05
3.61	0.05
3.37	0.05

8. 4-Ethylhexanoic

d, A.	I/I_1
18.2	1.00
16.5	0.05
14.4	0.50
12.5	0.50
11.3	0.15
10.1	0.15
9.21	0.40
8.56	0.35
7.22	0.10
6.70	0.10
6.05	0.40
5.08	0.20
4.77	0.10
4.60	0.05
3.68	0.05
3.53	0.20
3.33	0.20
3.22	0.05
3.07	0.20
2.87	0.05
2.81	0.20
2.64	0.05
2.54	0.10
2.46	0.10

(*Contd.*)

8. 4-Ethylhexanoic

d, A.	I/I_1
2.32	0.05
2.29	0.05
2.24	0.05
1.83	0.05
1.79	0.05
1.76	0.05
1.59	0.05

9. 2-*n*-Propylhexanoic

d, A.	I/I_1
14.5	1.00
11.8	0.50
8.84	0.20
7.13	0.30
5.74	0.10
4.95	0.05
4.46	0.05
3.92	0.05
3.53	0.05
2.71	0.05

10. 2-Isopropylhexanoic

d, A.	I/I_1
14.2	1.00
12.1	0.20
11.4	0.30
9.94	0.50
7.72	0.10
6.95	0.20
6.28	0.30
4.97	0.10
4.75	0.20
4.27	0.10
3.90	0.05
3.73	0.05
3.46	0.10
3.17	0.10
2.82	0.05
2.74	0.05
2.70	0.05
2.64	0.10
2.50	0.10
2.34	0.05
2.21	0.05

11. 3-*n*-Propylhexanoic

d, A.	I/I_1
17.6	1.00
13.6	0.10
12.1	0.60
10.4	0.40
9.13	0.30
8.63	0.20
8.01	0.10
7.32	0.20
6.51	0.15
5.73	0.15
5.35	0.15
4.63	0.10
4.35	0.15

(*Contd.*)

11. 3-*n*-Propylhexanoic

d, A.	I/I_1
3.88	0.20
3.70	0.30
3.38	0.20
3.05	0.10
2.94	0.15
2.86	0.15
2.72	0.10
2.62	0.05
2.56	0.05

12. 2-*n*-Butylhexanoic

d, A.	I/I_1
14.4	1.00
13.6	0.95
11.7	0.40
10.7	0.40
8.24	0.50
7.62	0.20
7.32	0.20
6.78	0.30
6.11	0.10
5.45	0.20
5.30	0.20
4.99	0.05
4.44	0.10
4.19	0.05
4.04	0.05
3.68	0.10
3.57	0.10
3.34	0.10
3.06	0.10
2.95	0.10
2.73	0.10
2.63	0.10
2.51	0.10

13. 2-*sec*-Butylhexanoic

d, A.	I/I_1
14.3	1.00
11.4	0.30
8.91	0.10
8.31	0.10
7.78	0.20
7.18	0.10
3.59	0.05
2.74	0.10

14. 3-*n*-Propylheptanoic

d, A.	I/I_1
17.8	1.00
14.6	0.10
12.3	0.50
10.9	0.20
9.72	0.05
8.63	0.05
5.67	0.05
4.61	0.05
3.99	0.10
2.70	0.05

being used in the preparation of aluminum soaps, and means of identifying these acids are of value for this reason. Changes in long spacing in an isomeric series when the position of branching in the carbon chain is changed are also of interest.

PREPARATION OF SILVER SALTS

The acids were prepared by the Bureau of Mines and were obtained from the Army Chemical and Radiological Laboratories.

Table II. Silver Analysis and Three Most Intense Diffraction Lines of Silver Salts

Pattern No.	Three Strongest Lines, A. 1st	2nd	3rd	Parent Acid	% Silver Found	Calcd.
1	12.8	9.74	8.37	2-Methylhexanoic	45.9	45.5
2	15.5	7.72	12.1	3-Methylhexanoic	45.8	45.5
3	15.6	7.78	5.16	4-Methylhexanoic	45.2	45.5
4	15.8	7.78	5.20	5-Methylhexanoic	45.1	45.5
5	16.5	12.7	8.13	3,5-Dimethylhexanoic	42.7	43.0
6	13.6	11.2	6.74	2-Ethylhexanoic	43.2	43.0
7	16.7	11.2	8.35	3-Ethylhexanoic	43.1	43.0
8	18.2	14.4	12.5	4-Ethylhexanoic	43.0	43.0
9	14.5	11.8	7.13	2-*n*-Propylhexanoic	40.8	40.7
10	14.2	9.94	11.4	2-Isopropylhexanoic	40.7	40.7
11	17.6	12.1	10.4	3-*n*-Propylhexanoic	40.5	40.7
12	14.4	13.6	8.24	2-*n*-Butylhexanoic	38.8	38.7
13	14.3	11.4	7.78	2-*sec*-Butylhexanoic	38.7	38.7
14	17.8	12.3	10.9	3-*n*-Propylheptanoic	38.5	38.7

The position of branching was determined by the method of synthesis. Carbon-hydrogen analysis and neutralization equivalents were reported. Agreement between the calculated and experimental values was excellent. Freezing point data were used to determine mole per cent purity in several cases. The 2-isopropyl-, 2-*n*-butyl-, 3-*n*-propyl-, 4-ethyl-, and 5-methylhexanoic acids were better than 95 mole % pure. The 2-ethyl- and 3-methylhexanoic acids were 92 and 89 mole % pure, respectively.

The silver salts were prepared by the method used by Matthews, Warren, and Michell (*3*). The analytical results for the salts are given in Table II.

X-RAY APPARATUS AND TECHNIQUE

X-ray diffraction measurements were made with a General Electric XRD-3 instrument using copper $K\alpha$ (λ = 1.5418 A.) radiation with a nickel filter. The samples were ground to a fineness of 200 mesh and were mounted in the usual manner in a Debye-Scherrer camera. The sample was rotated continuously throughout the exposure time of 20 hours. The intensities were estimated by means of a film calibrated according to the recommendations of Klug and Alexander (*1*). The three most intense diffraction lines of the silver salts are listed in Table II, while the complete patterns for each salt are listed in Table I.

DISCUSSION

As can be seen from Table I, the long spacing in any isomeric series varies as the position of branching changes. In the methyl, ethyl, and propyl hexanoate series the long spacing increases as the group is moving farther from the carboxylate group. The greatest change in long spacing occurs when the group is moved from the 2 to the 3 position. The long spacing remains approximately the same whether an *n*-propyl or isopropyl group is in the 2 position. The long spacings of the silver salts of 2-*n*-butyl- and 2-*sec*-butylhexanoic acids are also the same.

ACKNOWLEDGMENT

This study was conducted under contract between the Chemical Corps, U. S. Army, and Rensselaer Polytechnic Institute.

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RECEIVED for review November 8, 1955. Accepted June 30, 1956.

Identification of Ketones by Catalytic Wolff-Kishner Reduction of Hydrazones and Mass Spectrometry

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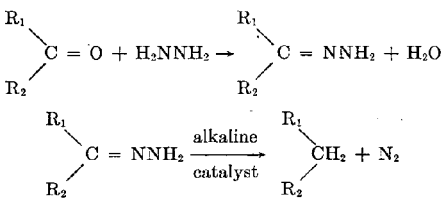
A method is presented for the identification of some aliphatic ketones, based on the Wolff-Kishner reduction of these materials to alkanes. Mass spectrometric characterization of the carbon chain configuration of the alkane leads to identification of the original ketone, in many cases. Identification of C_7 and C_8 ketones, including the semiquantitative analysis of their isomeric mixtures, has been achieved in synthetic samples and experimental products. The procedure developed for conducting the Wolff-Kishner reduction is also believed to be novel.

KETONES are generally identified by comparing the physical and chemical properties of the ketone to be identified with the corresponding properties of pure, known ketones. The determination of melting points of derivatives, infrared absorption spectroscopy, mass spectrometry, adsorption, and paper chromatography are techniques that rely upon such comparisons for identification. However, in experimental work reference samples of the ketones in question are not always readily available. Such a situation occurred during an investigation of the synthesis of C_7 and C_8 aliphatic ketones, and led to the development of an apparently novel identification method based on the Wolff-Kishner reduction of ketones to hydrocarbons. C_7 and C_8

ketones are reduced to the corresponding alkanes. With a few exceptions, the carbon chain configuration of the alkanes can be more readily determined by standard mass spectrometric methods than the configuration of the corresponding ketones, because of the more extensive catalog of data available for the alkanes. Although the position of the carbonyl group is not established, the carbon chain configuration along with knowledge from the method of synthesis will usually enable exact identification. The need for reference ketones is markedly reduced by this conversion of ketones to alkanes, because pure alkanes are relatively much more available as reference materials than ketones, particularly in the C_7 and C_8 range. Semiquantitative analysis as well as identification has also been achieved for mixtures of C_7 and C_8 ketone isomers, which differ in their carbon chain configuration rather than in the position of the carbonyl group. The method is, of course, not applicable to mixtures of ketones where the position of the carbonyl group is a critical factor.

In the Wolff-Kishner reduction of aldehydes and ketones the oxygen atom of the carbonyl group is replaced by two atoms of hydrogen. The reduction is carried out by heating the semicarbazone, hydrazone, or azine derivative of a carbonyl compound in the presence of an alkaline catalyst, as follows:



Todd (*6*) has discussed in detail the reaction mechanisms and variables as well as experimental procedures devised by various investigators. Kishner (*4*) dropped hydrazones at a slow rate upon hot potassium hydroxide mixed with platinized porous plate. Wolff (*8*) heated hydrazones or semicarbazones in a sealed tube to about 180° C. in the presence of sodium ethoxide. Huang-Minlon (*3*) was able to conduct the reaction in an open flask with the aid of a high boiling solvent, triethylene glycol.

To meet the need for a semiroutine procedure, the authors developed a technique in which the ketones are reduced by a continuous operation. Aside from the utility offered by this procedure for the identification of ketones, as discussed below, the procedure may also be viewed as a new technique in organic synthesis. The ketone is made to react with hydrazine in pyridine for 1 hour at 60° C. to form the corresponding hydrazone. The reaction mixture is fed at a steady rate into a reaction tube maintained at 200° C. and containing a Porocel catalyst, previously treated with potassium hydroxide. A constant flow of nitrogen through the system forces the crude product out of the reaction tube. An ice trap collects the product, which is separated from the pyridine by washing with aqueous hydrochloric acid. Adsorption chromatography over silica gel further purifies the alkane to remove polar side products, which generally do not exceed 1% of the alkane. The alkane is thereby isolated and subsequently characterized by mass spectrometric methods.

APPARATUS

A schematic diagram of the apparatus is given in Figure 1. The borosilicate reaction tube was equipped with a pyrometer accurate to ±5° C. The dimensions were not considered critical, except that the entire catalyst zone was heated and the thermocouple well placed near the top surface of the catalyst. The reaction tube was heated by a tubular electric furnace, mounted vertically and capable of uniformly maintaining a temperature of 200° C. Oxygen present in the nitrogen was removed by passing the nitrogen through a borosilicate glass tube containing metallic copper wire held at a temperature of 300° to 350° C. by a tubular electric furnace.

Catalyst. The catalyst was activated Porocel (2/4 mesh, 30% volatile matter) obtained from the Attapulgus Clay Co., Philadelphia, Pa. The activated Porocel catalyst was ground to 8/12 mesh and 35 cc. of the ground Porocel was placed in 100 ml. of 40% aqueous potassium hydroxide solution for 1 hour at room temperature. At the end of this time, the alkali was decanted and the treated Porocel dried for 4 hours at 110° C. under a nitrogen atmosphere.

PROCEDURE

The apparatus was assembled as indicated in Figure 1, except that the sample flask was omitted and the plug was connected directly to the cap. The exit tube of the reactor was immersed in 25 ml. of 6*N* hydrochloric acid solution contained in a 60-ml. separatory funnel placed in a steel beaker filled with ice. The neck of the reactor was plugged with a small amount of glass wool and sufficient catalyst was added just to cover the thermocouple well. Oxygen-free nitrogen was passed through the system at a rate viewed in the separatory funnel of approximately two bubbles per second. The tubular electric furnaces were adjusted so that the temperature of the reactor was 200° ± 10° C., while the tube containing the copper wire was at a temperature of 300° to 350° C. Fresh catalyst was used for each run.

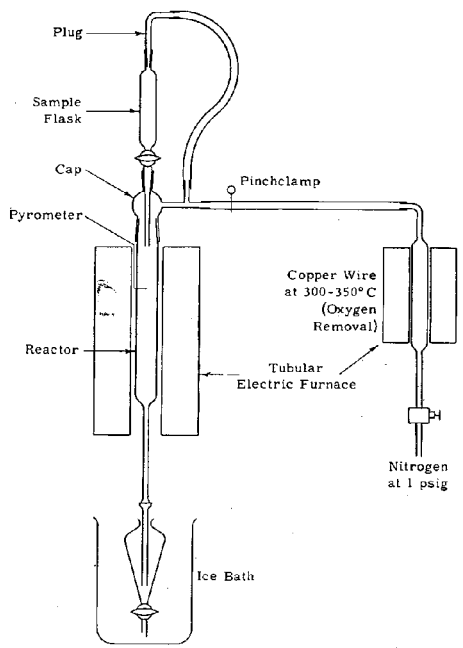


Figure 1. Diagram of Wolff-Kishner reduction apparatus

Approximately 26 mmoles of a ketone were added to the sample flask, which contained 15 ml. of pyridine and 3 ml. of hydrazine (95+%, Eastman). The flask was placed in an oven at 60° C. for 1 hour, and then was inserted between the plug and the cap of the reactor. The stopcock of the flask was adjusted so that the reaction mixture was fed dropwise to the reactor at such a rate that the temperature of the reactor was maintained at 200° ± 10° C. After proper adjustment was achieved, the nitrogen flow was cut off with the pinchclamp. When all of the reaction mixture had been consumed, the system was again purged with nitrogen. The reaction product, contained in the separatory funnel, was washed several times with 1*N* hydrochloric acid, and the upper hydrocarbon phase was retained. One milliliter of the washed product was purified by adsorption chromatography over silica gel by the fluorescent indicator adsorption method (*2*). In this technique, the saturated hydrocarbons form a nonfluorescent zone in ultraviolet light. This zone is the fastest migrating under the

Table I. Application of Wolff-Kishner Reduction Method to Some C_7 and C_8 Ketones

Ketone Tested	Ketone Structure	Carbonyl Value (2), Wt. %	Purity by Adiabatic Calorimetry (7), Mole %	Wolff-Kishner Reduction Product: Hydrocarbon identified	Yield, %	Purity, mole %[a]
4-Methyl-2-hexanone	$CH_3C(=O)CH_2CH(CH_3)CH_2CH_3$	98.4	99.4	3-Methylhexane	79	99.8
5-Methyl-3-hexanone	$CH_3CH_2C(=O)CH_2CH(CH_3)CH_3$	99.4	99.7	2-Methylhexane	86	100.0
3,4-Dimethyl-2-hexanone	$CH_3C(=O)CH(CH_3)CH(CH_3)CH_2CH_3$	99.2	..	3,4-Dimethylhexane	87	99.5
5-Methyl-3-heptanone	$CH_3CH_2C(=O)CH_2CH(CH_3)CH_2CH_3$	100.0	>99.3	3-Methylheptane	76	99.5

[a] Mass spectrometry.

conditions of the method. This fraction is collected until the first appearance at the exit of the chromatographic tube of fluorescence, which may be yellow, blue, or reddish brown.

A modified Westinghouse 90° sector mass spectrometer employing 1000-volt positive ion acceleration and magnetic scanning recorded the mass spectra of the saturated hydrocarbon products. The ionizing electron energy was 75 electron volts.

Mass spectral identification of the carbon skeleton configuration is based on the dissimilarities in the fragmentation patterns of isomeric molecules. The probability of the formation of a fragment ion of a particular mass-charge ratio on electron impact is strongly dependent on the structure of the parent molecule. Fragmentation patterns of the isomeric alkanes, therefore, are characteristic and sufficiently different to permit, in general, an accurate resolution of the mass spectrum of a mixture of isomers, particularly if the number of isomers present does not exceed two or three. Accordingly, the unicomponent alkane products of the Wolff-Kishner reduction were readily identified by comparison of their spectra with those of pure reference alkanes. In addition, the detection of trace impurities at concentrations equal to or greater than 0.2 mole % is usually possible. Relative concentrations of isomers in polycomponent products were determined from simultaneous equations involving ion intensities at mass-charge ratios chosen to minimize the errors in the estimation of the individual isomers. Such calculations have been described, for example, by Brown and associates (*1*).

RESULTS AND DISCUSSION

Several pure C_7 and C_8 ketones were tested by the method (Table I). In each case an alkane was obtained which corresponded in carbon chain configuration with the original ketone. No indications of isomerization during the reduction were observed. These results are in excellent agreement with those established by the adiabatic calorimetric method used. In general, the alkanes were isolated in 80 to 90% yield. Side reactions appeared to be small, as evidenced by the small amounts of polar compounds, less than 1% of the alkanes, detected during the chromatographic purification of the product.

Semiquantitative analysis of mixtures of C_7 and C_8 ketone isomers was possible when the isomers had different carbon chain configurations. A few examples of such analyses are shown in Table II. The mixture of 5-methyl-3-hexanone and 2,3-dimethylpentanone was an actual experimental product which was believed to be a pure sample of 5-methyl-3-hexanone. Application of the Wolff-Kishner reduction method showed that the material consisted of an isomeric mixture. Although other isomers could have been present without being detected, the method of synthesis used eliminated this possibility.

An additional interesting application of the method is shown in Table III. During some experimental investigations a fractional distillation was carried out on a mixture of 5-methyl-3-hexanone (boiling point 136° C.) and 4-methyl-2-hexanone (boiling point 141° C.). Determination of the compositions of the various distillation cuts was hampered by the lack of pure samples of these ketones. However, the compositions could be followed very nicely by the Wolff-Kishner reduction method.

Table II. Analysis of Mixtures of C_7 and C_8 Ketone Isomers by Wolff-Kishner Reduction Method

Mixture	Added, Wt. %	Found, Wt. %
5-Methyl-3-hexanone[a]	67.3	63.3
4-Methyl-2-hexanone	32.8	36.6
5-Methyl-3-hexanone[b]	..	84.4
2,3-Dimethylpentanone	..	15.6
5-Methyl-3-heptanone[a]	80.0	84.4
3,4-Dimethyl-2-hexanone	19.7	16.1

[a] Synthetic mixture.
[b] Experimental product.

Table III. Analysis of Fractions from Distillation of Mixture of C_7 Ketone Isomers by Wolff-Kishner Reduction Method

Fraction	5-Methyl-3-hexanone[a], Wt. %	4-Methyl-2-hexanone[b], Wt. %
5	68.3	31.7
7	35.7	64.3
9	6.6	93.4

[a] Boiling point 136° C.
[b] Boiling point 141° C.

ACKNOWLEDGMENT

The authors wish to express their appreciation to D. P. Stevenson and F. T. Weiss for their guidance and suggestions.

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RECEIVED for review April 5, 1956. Accepted July 16, 1956. Seventh Annual Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., March 1956.

Application of X-Ray Emission Spectrography to Air-Borne Dusts in Industrial Hygiene Studies

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The ability of x-ray emission spectrography to determine the heavy metal elements quantitatively in the range of 1 to 100 γ in the absence of interferences has been applied to industrial hygiene studies. Air-borne dusts containing heavy metals may constitute a health hazard in the manufacture, processing, and packaging of catalysts, ores, and pigments. The atmosphere is sampled by drawing the air at a known rate through a glass fiber filter disk which catches the air-borne dust. The dust is imprisoned on the disk by application of an acrylic resin spray to protect the sample during shipment to the laboratory. The filter disk is examined directly in a Norelco x-ray emission spectrograph. Calibration curves, extending down to 1 to 5 γ, have been prepared for cobalt, chromium, iron, lead, mercury, nickel, platinum, vanadium, and zinc. Typical examples are air-borne dusts from catalysts (platinum, vanadium), ores (cobalt), and pigments (chromium). Extension to many other elements is feasible.

THE ability of x-ray emission (fluorescence) spectrography to determine various elements quantitatively in the range of 1 to 100 γ in the absence of interferences has been applied to industrial hygiene studies. Air-borne dusts containing certain elements may constitute a health hazard in the manufacture, processing, or packaging of catalysts, ores, and pigments. These dusts may be sampled with simple apparatus, and analyzed by use of the x-ray spectrograph, in an accurate and rapid procedure.

In 1954, Pfeiffer and Zemany (*6*) demonstrated that several heavy metals could be detected in microgram quantities, using zinc as an example. Cavanaugh (*2*) described the determination of trace amounts of elements by x-ray emission by use of thin plastic films on which the elements had been deposited following a chemical separation. Microanalysis using x-ray emission spectrography has also been applied to thin films of ion exchange resins (*5*). Clark and Terford (*3*) employed x-ray emission spectrography in the determination of iron in air-borne foundry dusts, using a high-volume air sampler and long sampling periods.

The determination of very small amounts of material is desirable in industrial hygiene studies on air-borne dusts, not only for the detection of low concentrations of highly toxic materials, but also for shortening the sampling time, thereby permitting more samples to be taken. For the attainment of high sensitivities (low detectabilities) with the x-ray emission spectrographic method, it is highly desirable to present to the primary x-ray beam all the material from the atmosphere that was sampled. This may be accomplished by matching the area of the filter on which the air-borne dust is deposited to the effective area of the primary x-ray beam. Fortunately, the 2.4-cm. filter disk used in the compact air sampler (designed by J. B. Gisclard, and currently manufactured by the Davis Emergency Equipment Co., 45 Halleck St., Newark, N. J.) has an effective area fitting well with that of the primary x-ray beam.

SAMPLING PROCEDURE

For sampling the industrial atmosphere in operations of manufacturing, processing, or packaging of catalysts, ores, or pigments, air is drawn at a steady rate through the filter of the compact air sampler. This device, shown in Figure 1, consists of a centrifugal pump driven by an electric motor, a rotameter, trap, and the filter holder, connected by rubber tubing. As the maximum allowable threshold values for the elements in the air-borne dusts are expressed in milligrams per cubic meter for an 8-hour day, the amount of air sampled must be calculated from the flow rate (generally 10 liters per minute) and the time of sampling.

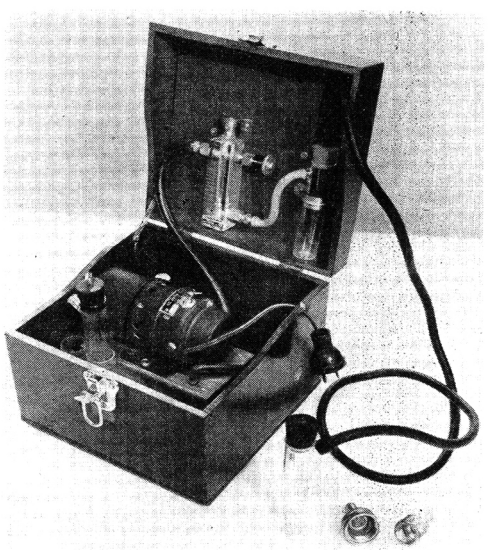

Figure 1. Compact air sampler

The glass fiber filter is held with rubber washers between the threaded sections of the filter holder, which resembles the sections of an ordinary garden hose connector. It is backed by a disk of No. 41 Whatman filter paper for strength. At the end of the sampling period, the filter holder is unscrewed, and the collected dust is imprisoned on the filter by spraying lightly with an acrylic spray from a small pressure can, while the air is still being drawn through the filter. The air flow is then shut off, and the disk is removed with a pair of tweezers and placed in a small glassine envelope or wrapped with aluminum foil. These envelopes are mailed or taken to the laboratory having the x-ray emission spectrographic equipment.

The filter with the imprisoned dust from the sample atmosphere is placed in an aluminum sample holder having a shallow cylindrical depression about 1 inch in diameter. This centers the filter in the primary x-ray beam of the Norelco x-ray spectrograph. The time to accumulate a predetermined number of counts is

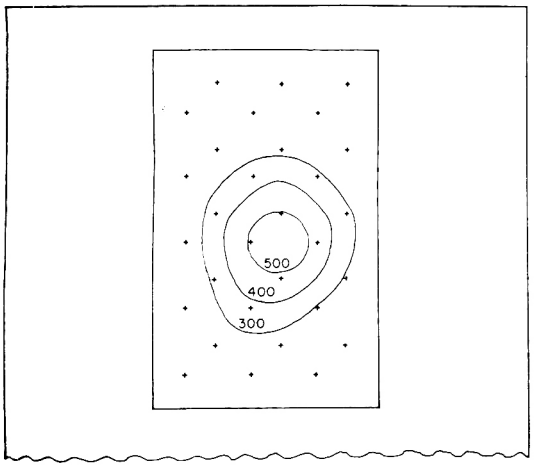

Figure 2. Contour map of sample area of holder

Contours are marked with counts per second. Plus signs indicate centers of copper square during counting. Handle of holder is off the bottom of figure

measured, and the number of counts per second is calculated. Counting times were always greater than 100 seconds. The "background" is measured by counting at a position 1 or 2 degrees 2 θ away from the analytical wave length of the element being determined. The background counting rate is subtracted from that at the analytical wave length The number of micrograms of the element being determined is read from a calibration graph of counts per second versus micrograms.

PREPARATION OF CALIBRATION CURVES

Some difficulties were encountered initially in the preparation of standards for construction of calibration curves. It was difficult to obtain a uniform deposit by evaporation of different volumes of solutions of known concentration onto an aluminum foil or filter disk. The filter disks were rendered nonabsorbent by spraying with the acrylic resin before the sample was deposited, and sprayed again for preservation. For a given set of standards, it was found necessary to evaporate equal volumes of standard solutions of varying concentrations to obtain a uniform deposit. Standards are preserved for future use. A standard is reexamined for each set of analyses.

The uniformity of the x-ray beam was investigated in a manner similar to that of Davis and Hoeck (*4*).

A square of copper, about 3 mm. on a side, was counted in a sample holder with a grid of lines drawn upon it. Readings were taken at 30 locations. These counting rates were located on a graph ten times larger than the grid of the sample holder. Intensities were interpolated by use of a Gerber variable scale (manufactured by the Gerber Scientific Instrument Co., Hartford, Conn.), and a "contour map" of counting rates (intensities) was prepared. The most intense beam was located approximately at the geometric center of the holder. Samples and standards were placed reproducibly in this area. The holder and contour map are shown in Figure 2.

The calibration points began to give counting rates below that expected from a linear plot of counts per second against micrograms of element when the total amount present exceeded 200 γ. This was believed due to a "piling up" of the deposit, so that reabsorption of the secondary x-rays and lack of penetration of the primary x-rays occurred. When amounts in excess of 200 γ were encountered, the samples were dissolved and determined colorimetrically. The highest point in Figure 3 illustrates this effect. As an alternative to the colorimetric procedure, an internal standard could be added to the dissolved sample and the desired elements could be determined by x-ray emission spectrography. Several papers have discussed the use of internal standards (*1*, *3*, *7*).

DETECTABILITIES

The detectability (sensitivity, or minimum amount detectable) was obtained by reading the amounts corresponding to a counting rate of 10 counts per second over background with the tube operated at 50 kv. and 50 ma. These data are summarized in Table I. The value of "10 counts per second over background" was selected as a limiting value which would be safely above the "noise" level. The detectabilities on other instruments operated under different conditions might be higher or lower than these values.

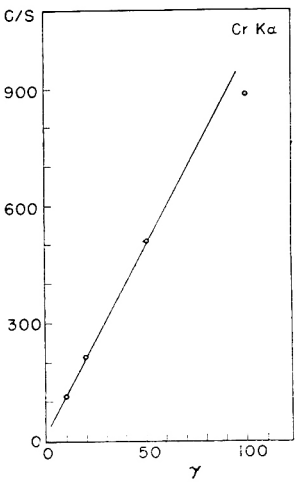

Figure 3. Calibration curve for chromium

Table I. Detectabilities of Elements Tested

(50 kv., 50 ma. NaCl crystal, helium flooded)

Element	Atomic No.	Line Used	Detectability, γ
Vanadium	23	$K\alpha$	1.5
Chromium	24	$K\alpha$	0.7
Iron	26	$K\alpha$	0.5
Cobalt	27	$K\alpha$	0.9
Nickel	28	$K\alpha$	0.9
Zinc	30	$K\alpha$	0.6
Platinum	78	$L\alpha_1$	6.6
Mercury	80	$L\beta_1$	7.8
Lead	82	$L\alpha_1$	7.0

The method was tested in the laboratory by creating synthetic air-borne dusts from a finely divided catalyst which contained iron. A stream of filtered air was passed through the powdered catalyst and then through the sampling filter. Filters were removed, sprayed, and analyzed in the x-ray spectrograph. In cases where sufficient amounts had been deposited, the filters were cooked in hydrochloric acid and a colorimetric determination was performed by the *o*-phenanthroline method. Good checks were obtained, as shown in Table II, for iron and for cobalt samples from air-borne dusts from processing of an ore concentrate. The cobalt samples were cooked in hydrochloric acid and determined colorimetrically as cobaltous chloride.

Generally, field samples tended to assay too low to be used as checks on the x-ray method.

Certain elements in the glass fiber disks may cause high backgrounds. The ultraviolet-emission spectrograph showed that iron, lead, nickel, and zirconium were present. The contribution to the total counts per second could be compensated for by a simple subtractive background correction, however, or use of filter paper disks that were free of these interfering elements.

Table II. Comparison of X-Ray Emission and Colorimetric Data

Element	Sample Description	X-Ray, γ	Colorimetric, γ
Cobalt	Ore concentrate, field sample	55	50
		140	200
Iron	Catalyst, synthetic dust	35	27
		89	89
		122	122

TYPICAL APPLICATIONS

During the past year, industrial atmospheres have been sampled for air-borne dusts produced in the manufacture and packaging of catalysts and analyzed for vanadium, platinum, or mercury. These samples were obtained in plants in New Jersey and West Virginia, and mailed to Stamford, Conn., for analysis. Results were reported by telephone on the day the sample was received. Samples obtained from ore processing were similarly transmitted from Utah. Blending operations, where pigments containing elements such as chromium or nickel are added to molding powders, have been so studied.

Extension of the method is feasible for any element in the periodic table which can be determined on the particular x-ray spectrograph used. Air-borne solid matter other than dust, such as smoke or smog, may be similarly sampled. If large amounts of air-borne material are trapped, where the x-ray method may become nonlinear (above perhaps 200 γ), the filter and samples may be processed to put the element sought into solution, where it may be determined by another technique, such as spectrophotometry, or by x-ray emission spectrography with use of internal standards.

CONCLUSION

The combination of a simple air-sampling device with x-ray emission spectrography results in a rapid, accurate, nondestructive technique for determining the heavy elements in air-borne dusts. Calibration curves are easy to construct, and the analysis is accurate and linear in the range of one to a few hundred micrograms.

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RECEIVED for review May 8, 1956. Accepted August 8, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, February 29, 1956.

Fluorometric Determination of Uranium

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The fluorometric method of determining uranium has been improved during several years of practical experience, in order to achieve a high degree of accuracy in the rapid, routine processing of a large, daily sample load. A modified Fletcher burner has been designed, on which 20 fluoride fusions are made at one time under reproducible fusion conditions. A fluorometric flux mixture of 2% lithium fluoride and 98% sodium fluoride is superior to other fluxes in sensitivity, precision, and convenience. The coefficient of variation of the method employing this new flux has been determined to be 0.7%. This constitutes at least a sixfold improvement in precision. The method has been proved to be rapid, accurate, and economical in almost 2 years of use at this laboratory, where uranium is determined in approximately 3000 samples each month.

THE Analytical Department of the Raw Materials Development Laboratory daily performs a large number of uranium determinations on a variety of products, both organic and inorganic. Volumetric, spectrophotometric, and fluorometric procedures are used, and the choice of procedure is governed by the accuracy required and the uranium content of the sample. Either the macrovolumetric procedure of Sill and Peterson (*26*) or a microvolumetric adaptation (*12*) is employed when the highest accuracy is desired. An adaptation of the thiocyanate spectrophotometric procedure (*19*) is used for routine samples containing more than 1 gram per liter or 5% U_3O_8. The fluorometric procedure described in this paper is usually employed for samples that contain too little uranium to be analyzed spectrophotometrically.

No attempt is made to review completely the extensive literature concerning the fluorometric determination of uranium. Several outstanding papers have been written on the subject, such as the article by Price, Ferretti, and Schwartz (*20*), which is probably the most comprehensive, and the excellent reviews by Rodden (*21, 22*). Since the publication of the last review article (*21*), several new fluorometers and modifications of older models for the measurement of the fluorescence of uraniferous phosphors have been described (*2-5, 11, 13, 15, 16*). Although several of these instruments have been excellently designed, all are custom-built and not commercially available. The use of aluminum nitrate as the "salting" agent for the separation of uranium by extraction into ethyl acetate, as first introduced by Grimaldi and Levine (*7*), has been widely adopted (*1, 10, 19, 28*). The critical factors in the preparation of the fluoride melt have been examined by Michelson (*17*), and a machine for preparing the phosphors has been described (*27*). Several review articles and manuals on methods of determining uranium (*8, 9, 23, 24*) have included versions of the fluorometric method of analysis.

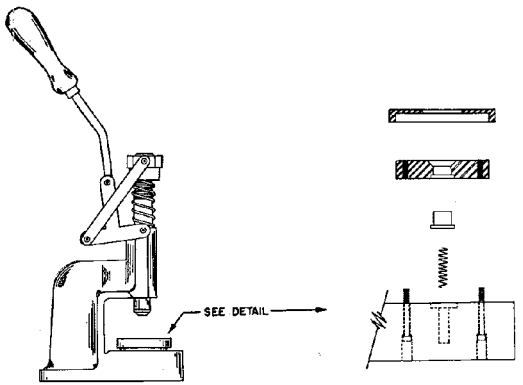

Figure 1. Die for forming platinum fusion dishes

The method for the fluorometric determination of uranium presented in this paper has evolved from several years of experience in uranium analysis. Continued improvements in fluorometer design during that time led to the development of the Galvanek-Morrison fluorometer *(18)*, which is used in the current method. Because this fluorometer is the only instrument of its type known to be commercially available, the authors have prepared a detailed description of their analytical method, so that others may conveniently profit from their experience. When the method was introduced a year ago at the Raw Materials Development Pilot Plant, Grand Junction, Colo., a marked increase in efficiency and accuracy was observed.

The purpose of this report is twofold: to present the fluorometric procedure, including not only the method of analysis, but also the various precautions and safeguards that are necessary to ensure continuous, accurate, routine analyses; and to describe a new fluoride flux, with data illustrating its superior sensitivity, precision, and convenience.

METHOD

A combination of dilution and solvent extraction is used to separate the uranium from possible interferences and quenchers. An aliquot of a liquid sample or of a solution of a solid sample is diluted and acidified so that the diluted sample contains approximately 0.01 mg. of U_3O_8 per ml. in a 5% nitric acid solution. One milliliter of the diluted sample is salted with saturated aluminum nitrate solution, and the uranium is extracted into 10 ml. of ethyl acetate. Aliquots of 0.1 ml. are removed and transferred onto pellets of 2% lithium fluoride–98% sodium fluoride flux in platinum dishes. The pellets are dried, fused, and allowed to cool, forming buttons which slide easily from the dish. The fluorescence of the fluoride button is measured in a Galvanek-Morrison fluorometer.

APPARATUS

The following special items may be used to equip a laboratory for fluorometric analysis.

A supply of 40-ml. vials, $4^1/_4$ inches long and 1 inch in diameter, and vial caps lined with aluminum foil. Although the vials are washed and re-used, the caps are so inexpensive that they may be discarded after use.

Two Machlett autopipets, 10-ml. capacity.

A variable-speed, box-carrier shaking machine with a wooden insert to hold the vials in a horizontal position.

Platinum Dishes. The dishes used in this laboratory are stamped from disks, 0.015 inch thick and 0.75 inch in diameter. The die (Figure 1) used in this laboratory for forming the dishes is custom-fabricated. A commercial dish former has recently been made available by the Jarrell-Ash Co., Newtonville, Mass. Best results are usually obtained by annealing the disks briefly at 850° C. prior to forming the dishes. The new dishes are cleaned by boiling in a 1 to 1 mixture of sulfuric and nitric acids, and are then rinsed thoroughly in water.

Drying Units. Double Chromolox radiant heater units (115 volts, 800 watts) are used in conjunction with a percentage timer (115 volts, 50 seconds, 20 amperes).

Pellet Maker. The stainless steel pellet maker is a custom-fabricated, modified version of an earlier model *(14)*. The construction details are shown in Figure 2. Adjustment of the pellet size is made by loosening knurled lock nut, *A*, turning the adjusting screw, *B*, clockwise for a smaller pellet or counterclockwise for a larger pellet, and securing the adjustment by turning the lock nut until it holds the adjusting screw tightly. In this laboratory a pellet weighing 0.40 gram has been found to be ideal in size.

Fusion Burner. A Fletcher burner is modified as shown in Figure 3. The lugs on the top of the burner are machined off. Pilot pins are embedded in the tops of the two front legs in order to center and hold an iron tripod ring $6^1/_4$ inches in diameter, with matching pilot holes, about 1 inch above the burner cap. Four circular, 60-mesh stainless steel screens, $4^1/_4$ inches in diameter, are placed in the burner bowl about $^1/_4$ inch below the burner cap to distribute the gas mixture evenly. A slotted tubular aluminum diffusion sleeve, $2^1/_2$ inches long and $1^1/_4$ inches in diameter, is placed in the burner to impinge the propane-air mixture sharply against the furthermost wall of the burner bowl, thus producing vigorous mixing of the gas and air. The original gas inlet is cut off about $^1/_2$ inch from the back leg of the burner, and a brass adapter with intakes for both gas and air is substituted.

Propane Regulator. The flow of propane gas to the fusion burner is measured with a precision-bore Flowrator, and is controlled with a needle valve.

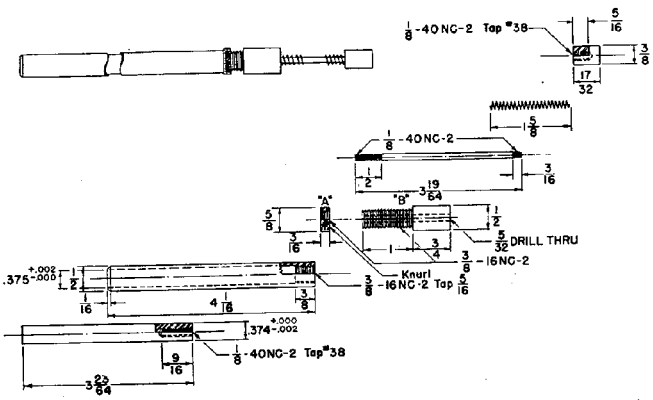

Figure 2. Construction details of pellet maker

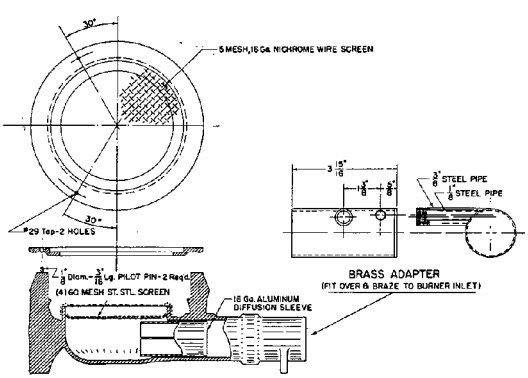

Figure 3. Modified Fletcher burner

Air Regulator. Air is supplied to the fusion burner by a Sutorbilt No. 2520 blower driven by a 1/3-hp., 1725-r.p.m. electric motor. As shown in Figure 4, flanges (*A* and *B*) are attached to both the intake and discharge openings of the blower to accommodate 3/4 × 3 inch pipe nipples, *C*. A 90° elbow, *D*, is connected to the air-intake nipple, and the open end of the elbow, *E*, is covered with 60-mesh stainless steel screen for air filtration. A 3/4 × 3/4 × 3/8 inch pipe tee, *F*, is connected to the air-discharge nipple. The air flows to the fusion burner through the 3/8-inch opening of the tee via a 3-inch nipple, *G*, and a Tygon hose, *H*. A custom-fabricated valve, *I*, shown in detail in Figure 5, regulates the flow of air to the fusion burner. A two-groove driving sheave, 3.75 inches in diameter, is attached to the motor shaft, *J*, and a two-groove driven sheave, 3.95 inches in diameter, is attached to the blower shaft, *K*. Two 35-inch A belts are used.

Nichrome wire gauze, circular sections, 4 1/2 inches in diameter, cut from 16-gage, 5-mesh Nichrome screen.

Galvanek-Morrison Fluorometer (*18*). In this laboratory the Mark III model (*6*) is used, but the instrument has recently been made available from the Jarrell-Ash Co., Newtonville, Mass., and the Engineering Equipment Co., Boynton Beach, Fla. A special fluoride button holder for use with the 2% lithium fluoride–98% sodium fluoride flux reported in this paper is shown in Figure 6. In this holder, the central depression in which the fluoride button is placed is highly polished to provide optimum reflectance, while the rest of the holder is coated with Aquadag colloidal graphite to minimize the reflection of light.

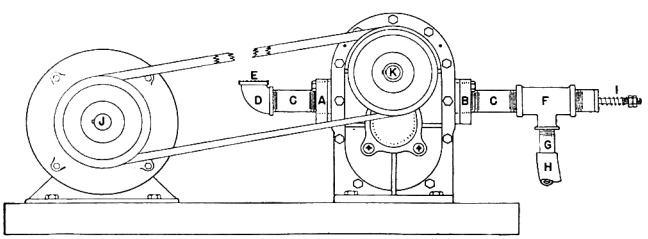

Figure 4. Air blower assembly

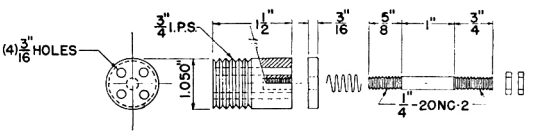

Figure 5. Detailed drawing of air regulator valve

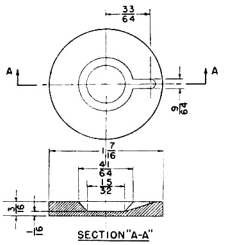

Figure 6. Aluminum receptacle for fluoride buttons

REAGENTS

The use of reagents that contain negligible uranium or other fluorescent impurities is essential. Even reagent grade chemicals are often so contaminated as to be unsuitable. In the list of reagents presented below, the manufacturer specified has been found in each case to supply a satisfactory grade of material.

Sodium Fluoride, reagent grade, Baker and Adamson Co., and lithium fluoride, reagent grade, Fisher Scientific Co., to be blended to form a mixture containing 2% lithium fluoride and 98% sodium fluoride.

The sodium fluoride is purchased in large quantity to assure uniformity from day to day. To select the best reagent available, representative samples of different production lots are obtained and checked for contamination and pelletizing characteristics. To be acceptable, the sodium fluoride must form pellets easily and cause negligible fluorescence. The fluorometer used in this laboratory normally shows a meter reading of about 80 units for 0.1 γ of U_3O_8 in the fluoride button, and a satisfactory batch of sodium fluoride will give a reading of 1 unit or less from a "blank" 0.40-gram button.

Ethyl Acetate, reagent grade. Ethyl acetate normally causes no interference; however, if contamination with either uranium or fluorescent organic material is suspected, it may be detected by preparing buttons from the usual amount of flux and 0.1 ml. of ethyl acetate. The fluorescence of buttons prepared from suitable ethyl acetate (and flux) should give meter readings of about 1 unit.

Aluminum Nitrate, reagent grade, J. T. Baker Chemical Co. Five pounds of the salt are dissolved in 1150 ml. of deionized water with heating. Before a solution batch is used, it is tested by preparing fluoride buttons according to the analytical procedure, but omitting the addition of sample. With a suitable batch of aluminum nitrate, the fluorescence from all ingredients used in preparing the fluoride buttons gives a meter reading of about 2 units.

Table I. Sample Size and Dilutions for Liquid Samples

Estimate, Mg. U_3O_8/Ml.	Sample Volume, Ml.	Dilution Volume, Ml.	Aliquot for Extraction, Ml.	Calculation Factor
	A. Aqueous Samples			
0.001–0.004	No dilution		2	0.00005
0.005–0.020	No dilution		1	0.0001
0.021–0.075	5	25	1	0.0005
0.076–0.120	5	50	1	0.001
0.121–0.275	5	100	1	0.002
0.276–0.330	5	200	1	0.004
0.331–0.70	1	50	1	0.005
0.71 –1.5	1	100	1	0.01
	B. Organic Samples			
	For Carbonate Strip			
<0.021	2	None	2	0.00025
0.021–0.075	1	None	2	0.0005
0.076–0.275	1	None	1	0.001
0.276–0.70	1	5/25	1	0.005
0.71 –1.5	1	5/50	1	0.01

RECOMMENDED PROCEDURE

Sample Preparation. Dilute all aqueous samples according to the estimated concentration, so that 1 ml. of the diluted sample contains approximately 0.01 mg. of U_3O_8. Some of the dilutions commonly used in this laboratory are listed in Table I. As the diluted sample should contain 5 volume % free nitric acid, basic samples must be neutralized before the proper amount of nitric acid is added. Add dropwise 30% hydrogen peroxide to any samples known to contain reduced uranium, until the permanent light yellow color characteristic of uranium(VI) is obtained.

Organic samples, such as those from solvent extraction studies, are best treated by stripping the uranium into aqueous carbonate solution before proceeding with the dilution and extraction. Pipet a suitable volume of the sample (Table I) into an extraction vial, and add 2 to 3 ml. of carbon tetrachloride to make the organic layer heavier than water. Add 10 ml. of 5% (w./v.) sodium carbonate solution, cap the vial, agitate on the shaker for 1 to 2 minutes, and centrifuge to separate the phases completely.

Treat the resulting carbonate solution according to the procedure described above for an aqueous sample.

Solids are treated by one of three methods: perchloric-nitric acid digestion, hydrofluoric-nitric acid digestion, and sodium hydroxide–sodium peroxide fusion. The first method is the most rapid and convenient of the three, but sometimes gives low results. The second method is most applicable to high-silica ores, while the third method is useful for ores containing carbonaceous matter. Each new ore sample is analyzed by all three methods, and subsequent samples of the same ore are analyzed by the method found to be most rapid and effective. Each solid is analyzed in duplicate according to the procedures outlined below. The proper sample sizes and dilutions are indicated in Table II.

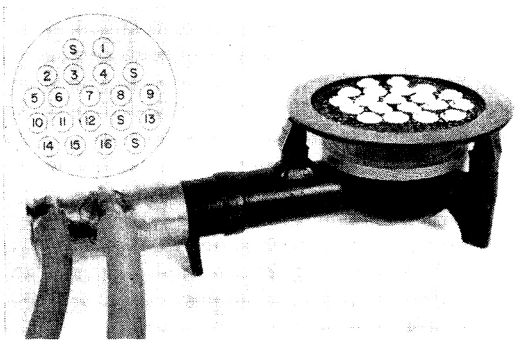

Figure 7. Burner assembly with pellets ready for fusion

Insert illustrates pattern for arranging platinum dishes. Locations marked S are for control standards; those marked with numbers are for samples

Perchloric-Nitric Acid Digestion. Weigh out the sample and add 40 ml. of nitric acid, 2 to 3 ml. of perchloric acid, and 30 to 40 ml. of water. Boil the mixture down to about 15 or 20 ml. Cool and dilute to the desired volume. Separate the solids from a portion of the sample and remove the desired aliquot for the subsequent ethyl acetate extraction.

Hydrofluoric-Nitric Acid Digestion. Weigh out the sample into a platinum or Teflon dish (*25*), wet with 2 to 3 ml. of nitric acid, and add 25 to 30 ml. of hydrofluoric acid. Evaporate to dryness, and dissolve in 20 ml. of 1 to 1 nitric acid. Heat again, and if the solution shows the presence of carbonaceous particles, add about 1 ml. of perchloric acid, evaporate to dense fumes, take up in water again, and dilute to the desired volume. Otherwise, proceed directly with the dilution.

Sodium Hydroxide–Sodium Peroxide Fusion. Weigh the sample into a nickel crucible and roast for 2 minutes at 750° C. Allow to cool. Add about 15 pellets of sodium hydroxide, and an amount of sodium peroxide approximately equal to the sample weight. Fuse the mixture at 750° C. for 7 minutes. Cool, and remove the solidified melt by leaching with hot water. Wash the crucible with 20 ml. of nitric acid, and add this wash and 20 ml. of hydrochloric acid to the water leach. Warm until the fusion mass has dissolved, evaporate if necessary, and dilute to the desired volume.

Extraction. Pipet the desired aliquot (see Tables I and II) of the diluted sample into an extraction vial. If no dilution was required and the sample is basic, acidify by adding concentrated nitric acid dropwise to the aliquot taken for extraction. Add approximately 15 ml. of aluminum nitrate reagent and exactly 10 ml. of ethyl acetate from an automatic pipet. Cap the vial, and agitate on the shaker for 1 to 2 minutes.

Preparation of Pellets. Place 20 washed platinum dishes on a Nichrome wire gauze (Figure 7) and dry under the Chromalox drying unit. The dishes must be dry, as placing flux pellets on wet dishes will eventually cause a severe attack on the platinum.

Place a 0.4-gram pellet of flux in each dish. Rotating the beaker containing the flux while filling the pellet maker will assure a uniform weight, and drawing the pellet maker across the bottom of the beaker will assure a flat bottom to each pellet.

From the ethyl acetate layer of each extracted sample, fill a 0.2-ml. graduated pipet and drain 0.1 ml. onto each of two pellets. Onto each of the four pellets reserved for control standards, pipet 0.1 ml. of ethyl acetate containing 1 γ of U_3O_8 per ml. (This ethyl acetate solution is prepared by extracting 1 ml. of a standard uranium solution containing 0.01 gram of U_3O_8 per liter according to the procedure described above under "Extraction.") Slowly volatilize the ethyl acetate from the pellets by placing the gauze holding the dishes under the drying units.

Fusion. Past experience has indicated that control of atmosphere and temperature plus continual uniformity of the fusion technique is mandatory for precise and accurate results.

Transfer the gauze holding the 20 platinum dishes and pellets to the iron tripod ring on the burner (see Figure 7). Fire the burner by opening the propane regulator valve to give a reading of about 5 units on the Flowrator with a gas line pressure of about 13 pounds. Switch on the air blower, and adjust the propane flow until the flame is of the proper height and temperature. Make this adjustment slowly, since the flame height diminishes as the gauze becomes heated. The procedure of adjusting the flame to the proper intensity requires experience before it can be done routinely. Periodically, the gas flow rates required to give temperatures of 850° and 900° C. should be determined with a thermocouple, until enough experience has been gained to set the flame by visual observation.

Fuse at about 900° C. for 2 minutes. Then decrease the temperature to 850° C. by increasing the gas flow, and continue the fusion for another minute. Turn off the air and the gas simultaneously. When the fluoride melts have solidified, remove the gauze and dishes from the burner and allow to cool for at least 15 minutes. (The fluorescence intensity increases for the first 15 minutes and then remains constant for about an hour.) The cooling rate affects the ultimate crystalline structure of the fluoride buttons, which in turn affects the fluorescence of the buttons. Therefore, it is necessary to follow the same procedure in cooling each set of dishes—i.e., place the gauze on a heat-resistant material away from drafts.

Table II. Sample Size and Dilutions for Solid Samples

Estimate, % U_3O_8	Sample Weight, Grams	Dilution Volume, Ml.	Aliquot for Extraction, Ml.	Calculation Factor
<0.001	2	100	2	0.00025
0.001–0.079	1	100	2	0.0005
0.080–0.20	1	100	1	0.001
0.21 –0.40	1	250	1	0.0025
>0.40	1	500	1	0.005

Measurement of Fluorescence. Instrument Adjustment. Optimum performance is obtained if the instrument is not shut off at the end of the work day but kept on continuously. When the instrument is on but not in actual use, it is advisable to have the sample slide pushed to the rear stop, so that the empty aluminum holder is in position rather than the fluorescent reference source. This avoids a fatigue effect in the phototube.

Pull the slide to the front stop. The artificial reference source is now under the ultraviolet light and the meter circuit is switched to the standard × 1 scale. Adjust the voltage applied to the phototube with the coarse and fine voltage controls so that the meter needle rests at full scale, or 100. This adjustment determines the applied voltage and therefore the sensitivity of the instrument. The meter reading for a fluoride button containing 0.1 γ of U_3O_8 is about 80 at this adjustment. Depress the zero-scale key and rotate the zero adjustment knob until the meter needle rests at zero. Repeat the voltage and zero adjustments alternately until no further changes are necessary.

Push the slide to the rear stop, and depress the × 0.1-scale key. Adjust the meter needle to zero with the background control. This background should remain constant during the day. If an increase in background is observed during use, contamination of the instrument is indicated. Any such contamination should be removed by cleaning rather than compensated for by background adjustment.

FLUORESCENCE MEASUREMENTS. The voltage and zero adjustments described above are checked before the fluorescence of each fluoride button is measured. The fluorescence intensities of the four 0.1 γ of U_3O_8 standard buttons are measured first and then those of the samples are measured in the order indicated in Figure 7.

Holding the platinum dish with forceps, tilt it so that the fluoride button slides into the aluminum holder. Push the slide to the rear stop. The lowest sensitivity ($\times$ 1000) scale is automatically in use when the slide is in this position. To read the fluorescence of the control standards, depress the key for the $\times$ 1 scale. For buttons of unknown intensity, depress the keys in increasing order of sensitivity so as not to drive the meter needle off scale. Pull the slide forward, and remove the button with forceps, cleaning any fluoride particles from the slide by aspiration.

Calculations. Obtain the reagent blank by preparing a set of fluoride buttons according to the recommended procedure but without the addition of any sample. (The daily reagent blank has been found to average about 2.0 $\pm$ 0.2.)

For each set of 20 buttons, average the readings of the four control standards and average the readings of all duplicate samples. Calculate the uranium content of the buttons according to the following relationship:

$$\text{Micrograms of } U_3O_8 \text{ in unknown} = \text{micrograms of } U_3O_8 \text{ in standard} \times \frac{\text{av. unknown reading} - \text{blank}}{\text{av. standard reading} - \text{blank}}$$

Obtain the final result by multiplying by the appropriate dilution factor given in Table I or II.

FLUOROMETER MAINTENANCE

The following maintenance procedure is recommended to keep the fluorometer functioning properly.

Fluorometer Slide. Each day the slide is removed from the instrument and coated with Aquadag if necessary. Anodized sample slides do not require treatment with Aquadag, but should be cleaned periodically.

Reference Source. Each day the reference source is examined to see that it is clean. The plastic sources supplied with commercial instruments may be cleaned by abrading the surface with fine emery cloth and wiping with a tissue dampened with alcohol. The use of paper sources was recently adopted in this laboratory. These sources are made by punching disks $^6/_8$ inch in diameter from stiff manila paper. The paper used in Reyburn No. 6AC shipping tags has a suitable fluorescence. Unlike uranium glass or plastic fluorescent sources, these paper sources are simply and economically made, are unaffected by temperature changes and exposure to ultraviolet light, and present no cleaning problem, since they may be discarded when dirty. A new paper source is usually inserted in the sample slide about every 3 days.

Ultraviolet Light Source. Two 4-watt, FT5, Sylvania Blacklite Blue lamps provide ultraviolet light in the instrument. It has been found necessary to replace the lamps three to four times a year, usually because of progressive diminution of light intensity. It is normally desirable to replace the lamps when the voltage required for full scale deflection with the reference source has increased to about 110% of the voltage initially required. If both lamps are not replaced at the same time, the probable difference in intensities will result in a nonuniform field of ultraviolet illumination. Occasionally, even though both lamps are replaced at the same time, a difference in intensities occurs after some use. In general, whenever fluorometer abnormalities are encountered, the ultraviolet light source should be suspected.

Vacuum Tubes. Tube replacement has been infrequent in the 4 years that the laboratory fluorometer has been used. On two occasions the 6SN7 tube in the measuring circuit has been replaced, and it has been necessary to recalibrate the measuring circuit. Directions for this calibration have been described (*6*). The tubes in the power supply have been replaced twice in 4 years. Rather than wait for malfunctioning of the power supply, it would appear preferable to replace power supply tubes annually.

CLEANLINESS

The fluorometric method of analysis is generally used in a laboratory or plant where uranium-bearing dust may be present in the atmosphere. Therefore, a room supplied with filtered air and used only for fluorometric analysis is desirable. To prevent the introduction of uraniferous dust, only personnel performing the fluorometric analysis should be allowed in this room. Each week the floors and benches in the room should be washed and about once a month the walls and overhead fixtures should be vacuum cleaned.

Glassware and other equipment used for this analysis should not be used for other purposes and should be scrupulously cleaned after each use. People working in the fluorometer room should not use protective hand creams, as these creams usually have a strong ultraviolet fluorescence.

EXPERIMENTAL

Many chemical compounds and mixtures have been used as the flux in the fluorometric determination of uranium. Flux selection is complicated by the many properties required of an acceptable flux. Fluxes currently in use may be placed in the following two categories:

High-Carbonate Fluxes. Low-melting (~600° C.) materials which after fusion and solidification are readily removed from the dish in a single button.

High-Fluoride Fluxes. High-melting (~1000° C.) materials which after fusion and solidification cannot be removed from the dish without fracturing the button.

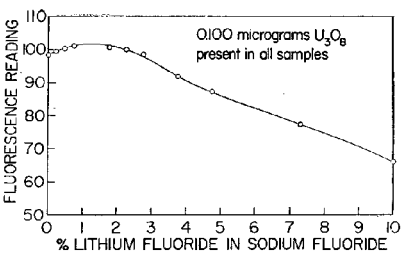

Figure 8. Effect of lithium fluoride on fluorescence of uranium in sodium fluoride

High-carbonate fluxes are exemplified by sodium fluoride–sodium carbonate–potassium carbonate mixtures containing 1 to 10% of sodium fluoride. In practice, the amount of this flux used per sample is approximately 2 to 3 grams, and the fusion is performed in a gold (*4*) or platinum (*8*) dish. The fusion dish plays no part in the measurement of the fluorescence intensity of the button. All buttons are removed from the fusion dishes and their intensities are measured in a holder that reflects a minimum of the ultraviolet light. High-carbonate fluxes have the additional advantage of being usable in fluorometers employing either the reflection or the transmission principle.

Unfortunately, at least in reflection-type fluorometers, uranium fluorescence is lower in the high-carbonate fluxes than in the high-fluoride fluxes. Other objections to the use of high-carbonate fluxes are fluctuations between daily flux batches prepared from the same reagent lots, and necessity for moisture control because of the hygroscopic nature of the button. In addition, the presence of impurities in the sample being analyzed often causes more interference in high-carbonate fluxes than in high-

fluoride fluxes (*20*). Cadmium, for example, fluoresces only in the former. Also, quenching of uranium fluorescence is often more pronounced in high-carbonate fluxes.

Fluxes of the second type are exemplified by pure sodium fluoride. In practice, the amount of sodium fluoride flux used is approximately 0.4 gram, with the fusion performed in a platinum dish. This type of flux has the advantage of maximum enhancement of uranium fluorescence and therefore provides greater sensitivity than that afforded by other fluxes. Because of their smaller size, sodium fluoride pellets can be efficiently fused 20 at a time. On the other hand, the larger pellets of the high-carbonate fluxes are preferably handled only four to six at a time. Thus more uranium determinations can be performed per man-day by using a high-fluoride flux.

The principal limitation of the sodium fluoride flux is the necessity for careful control of the temperature and atmosphere during fusion in order to obtain analytical reproducibility. Another important limitation is the influence of the fusion dish on the fluorescence measurement. Because the button is not easily removed from the dish, the fluorescence of each button must be measured in its fusion dish, and accordingly the reflectivity of the dish affects the amount of light reaching the radiation detector. This reflectivity varies markedly from dish to dish, and constitutes an important source of error. The principal source of this variation is the presence of brown stains on the fusion dish, presumably formed by the high temperature reaction between the flux, the dish, and the atmosphere. To minimize these reflectivity differences, it is necessary after each use to clean and, where necessary, polish each dish by a tedious, carefully controlled, and detailed procedure.

The recommended procedure embodies a flux which offers all the advantages of both types of fluxes and none of their disadvantages. This flux is composed of sodium fluoride into which have been admixed small amounts of lithium fluoride. Although it has previously been reported (*20*) that fluxes containing lithium attack the platinum dishes, this chemical attack presumably takes place only with high-lithium fluxes because it is not observed with low-lithium fluxes such as those employed in the recommended procedure, and with fluxes containing as much as 10% lithium fluoride. It has been found that for measurements in the Galvanek-Morrison fluorometer with the recommended optics (*6*), flux compositions containing between 1 and 3% added lithium fluoride provide optimum performance. When the flux contains less than 1% lithium fluoride, the button is not readily removable from its dish, while at concentrations higher than 3% lithium fluoride, the fluorescence per unit weight of uranium is inferior, as shown in Figure 8. Accordingly, a 2% lithium fluoride–98% sodium fluoride mixture has been selected as the flux composition.

A temperature of 850° to 900° C. has been found to be adequate for the fusion of the mixed-fluoride flux. The resultant button is readily removed from its dish, so that the fluorescence of each button is measured in the same holder and variation in reflectivity is eliminated. A polished aluminum receptacle has been built into the fluorometer slide to hold the button during fluorescence measurements. The aluminum receptacle has two primary functions. First, it acts as a reflector of induced radiation, thereby increasing the sensitivity of the analytical method by the additional fluorescence reflected into the radiation detector. Secondly, it provides a fixed and uniform amount of reflection of the ultraviolet light used to induce uranium fluorescence, thereby providing a constant background.

As the new flux allows all fluorescence measurements to be made without the fusion dish, the necessity for carefully controlled cleaning and polishing of fusion dishes is eliminated. The dishes are simply rinsed thoroughly in water and re-used. Even after a fluoride button containing as much as 10 γ of U_3O_8 has been prepared in a dish, no contamination of the dish has been noted after a thorough water wash. After 2 or 3 months of use, the dishes may be given a thorough polishing to preserve the platinum.

The analytical precision afforded by the mixed-fluoride flux has been found to be much superior to that afforded by any other known flux. The frequency histograms in Figure 9 clearly illustrate the excellent reproducibility obtainable. This figure was prepared from fluorometric data obtained from 500 fused pellets, each containing 0.1 γ of U_3O_8. Of these pellets, 250 were prepared from pure sodium fluoride and 250 were prepared from the mixed-fluoride flux. The fluorescence of the sodium fluoride buttons was of necessity measured in the fusion dishes; the fluorescence of the mixed flux buttons was measured in the aluminum receptacle. The instrument was adjusted for measurement of the fluorescence of the sodium fluoride buttons so that approximately the same average meter reading was obtained as with the mixed-fluoride buttons. As shown, determinations with sodium fluoride flux exhibit a much greater dispersion than corresponding determinations with the mixed-

Table III. Analyses of Standard Ore Samples

Ore Sample	U_3O_8 Present[a], %	U_3O_8 Determined, %
NBL 3	3.32 ± 0.08	3.37 3.37
NBL 4	0.177 ± 0.007	0.170 0.171
NBL 5	0.113 ± 0.008	0.120 0.118
AEC 8	0.064 ± 0.002	0.0646 0.0642
AEC 10	0.399 ± 0.009	0.396 0.397

[a] Certified average value and range limits at 95% confidence level.

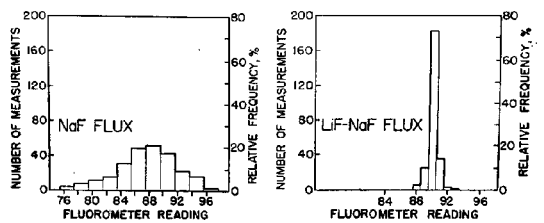

Figure 9. Comparison of reproducibility obtained with 100% sodium fluoride and 2% lithium fluoride–98% sodium fluoride

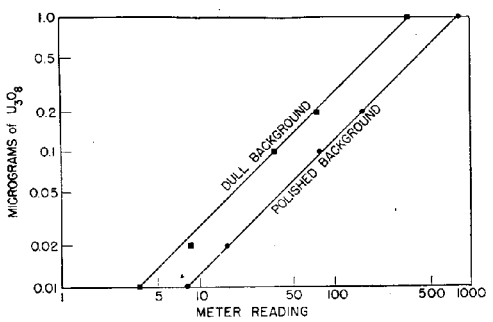

Figure 10. Calibration curves showing increased sensitivity with polished aluminum receptacle

fluoride flux. The relative variability (coefficient of variation) of the method employing the mixed-fluoride flux is 0.7%, compared with 4.7% when sodium fluoride is used as the flux.

In order to illustrate the increase in sensitivity afforded by measuring the fluorescence of the buttons in a polished holder, calibration curves were prepared by measuring the fluorescence of a series of mixed-flux buttons containing known amounts of uranium. These buttons were measured once in a polished aluminum receptacle, and again in a nonreflectant, anodized aluminum holder. As can be seen from the calibration curves shown in Figure 10, a higher sensitivity is obtained with the highly reflectant background.

The accuracy of the fluorometric method employing the mixed-fluoride flux is illustrated by analyses of five standard ore samples shown in Table III. All analyses were performed in such a manner that each fluoride button contained approximately 0.1 γ of uranium. In all cases the observed U_3O_8 content was within the limits of uncertainty of the certified U_3O_8 content.

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RECEIVED for review March 8, 1956. Accepted August 15, 1956. The Raw Materials Development Laboratory is operated by the National Lead Co., Inc., for the U. S. Atomic Energy Commission. Work carried out under Contract No. AT(49-6)-924.

Multiple Thickness Cell Assemblies

Application to Ultraviolet Spectrophotometry

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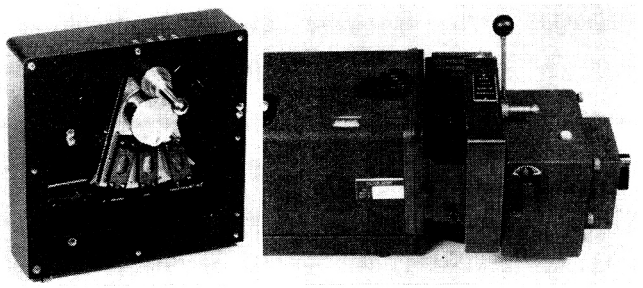
The use of a single absorption cell with a fixed length is often inefficient for making absorption measurements. Much time can be saved by using a multiple thickness cell assembly consisting of a group of individual cells of different thickness all filled with the same sample. The absorbances are then measured using the most appropriate cell thickness for the particular spectral region. This arrangement eliminates the need for preparing a different dilution of the sample for each region. Three cell assemblies of this type are described. These include a gas cell for the Beckman DU spectrophotometer and liquid cells for the Cary and the Beckman spectrophotometers.

SPECTROPHOTOMETERS are useful for measuring absorbances through only a limited range of values. If the absorbance falls outside this range it becomes necessary to adjust either the cell length or the sample concentration. Most ultraviolet absorption measurements are made using 1-cm. cells, with the sample concentration adjusted accordingly. Hirt and King (*1*) have pointed out that in many cases it is advantageous to vary the cell length rather than the sample concentration, and they have described the use of variable-length cells for this application. Another approach which has been useful in this laboratory is the use of a so-called multiple cell. This consists of a group of cells, each with a different length. The cells are all mounted together as a unit and arranged to fill in series so that all cells contain the same solution. The proper absorbance value is then obtained by merely selecting the appropriate cell for the measurement. One important advantage of this arrangement over variable-length cells is the higher accuracy that can be obtained in determining the thickness of the thinner cells. Also, one sample can be replaced with another sample somewhat more conveniently. On the other hand, the variable-length cell has the advantage of having only one cell blank, whereas the multiple cell has a separate cell blank for each cell.

Three different variations of multiple cells which have been used extensively in this laboratory are described below.

CELL CONSTRUCTION

Gas Cell for Beckman DU. The first multiple cell was designed for measuring the absorbance of dienes in the vapor state using



Unmounted Mounted COURTESY BECKMAN INSTRUMENTS

Figure 1. Triple gas cell assembly for Beckman DU spectrophotometer

the Beckman Model DU spectrophotometer. The experimental model constructed in this laboratory contained two cells with thicknesses of 1.00 and 0.05 cm. Later an improved commercial model with three cells was built by Beckman Instruments where it is designated as the triple gas cell assembly. The description which follows refers to this Beckman cell.

The individual cells are constructed from quartz plates, amalgamated lead gaskets, and suitable spacers, following the conventional design of infrared cells. Three such cells with thicknesses of 1.00, 0.10, and 0.01 cm. are mounted along the outer edge of a sector that rotates around a spindle located at the apex. One end of the spindle terminates in a male spherical joint. The sample passes in through this spherical joint, through each of the three cells in series and then into a reservoir with a volume of approximately 13 ml. The purpose of the reservoir will be described in a later section.

The cell assembly is mounted in a special compartment located between the standard cell compartment and the photocell housing of the Beckman spectrophotometer. A shift lever mounted on the spindle is used to rotate the sector to place any desired cell in the light path or to move the sector completely out of the beam. The cell is placed in the latter position when the sensitivity control on the spectrophotometer is adjusted or when conventional cells are used in the standard cell compartment. Detents are provided to position the cell in any one of these four positions. The nominal cell thicknesses are engraved on the cell compartment beside each position of the shift lever to indicate which cell is in the light path. Figure 1 shows the triple gas cell assembly with the side of the cell housing removed. The right half of Figure 1 shows the complete assembly mounted in the spectrophotometer.

Passageways are provided through the cell housing for circulating water from a constant temperature bath for thermostating the cell.

Liquid Cell for Cary. A multiple cell for liquids (and gases) was designed for the Cary spectrophotometer in this laboratory and was constructed by the Applied Physics Corp. The general arrangement and operation is similar to that of the Beckman gas cell. The major differences are the omission of the reservoir, the use of five cells with nominal thicknesses of 1.00, 0.30, 0.10, 0.03, and 0.01 cm., and provision for filling these cells with solutions. Both ends of the spindle on this cell terminate in male spherical joints. The solution passes in through one spherical joint, then through each of the five cells, starting with the thinnest cell and progressing to the thickest cell, and finally out through the other spherical joint. When the cell is rotated to the blank position, the entrance and exit connections to the individual cells are located so that all the cells fill without trapping air. Figure 2 shows the sector with the five cells.

The cell assembly is mounted in one end of a special compartment that replaces the standard compartment on the spectrophotometer. A quartz reference plate is provided, which can be moved into the reference beam for cancelling out cell reflections. The other end of the special compartment has space for sample and reference cells of conventional design. Cell lengths up to 5 cm. can be accommodated. Passages are provided for circulating constant temperature water for thermostating the cell compartment. The cell compartment in place is shown in Figure 3.

Liquid Cell for Beckman DU. A multiple cell for liquids (and gases), constructed in this laboratory for the Beckman Model DU spectrophotometer, again contains five cells with thicknesses of 1.00, 0.30, 0.10, 0.03, and 0.01 cm. However, these cells are mounted on a block that slides horizontally back and forth across

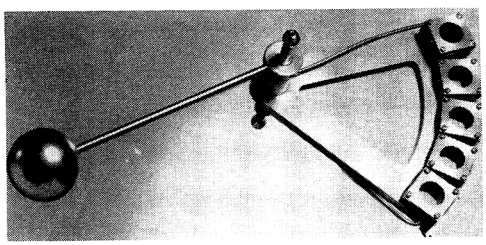


Figure 2. Sector for liquid cell Cary spectrophotometer

the light beam rather than on a sector such as is used with the cells described previously. A different method of positioning the cells is used in this case in order to provide a positive method of alternating the position of the cell assembly between some selected cell and the blank position. This is accomplished by moving the cell assembly up against stops at both ends of its travel. A fixed stop limits the forward motion of the assembly at a position where all of the cells are out of the light path. This provides for the blank position. Any one of five stops can be selected to limit the backward motion. Moving the cell assembly back against any stop positions the corresponding cell in the light path. These five stops are actuated by push buttons on top of the cell compartment. The corresponding cell thicknesses are engraved beside each button. No provision is made in this assembly for a reference quartz plate for cancelling out cell reflections.

The inlet and outlet tubes for filling the cells protrude through openings in the front of the compartment and terminate in male spherical joints. These tubes slide back and forth with the motion of the cell. The cells are again filled in series beginning with the thinnest cells, with the connecting lines arranged so as not to trap air in the cells. Passageways are provided through the bottom of the cell compartment for circulating water from a constant-temperature bath for thermostating the assembly.

The multiple cell compartment is mounted on the Beckman spectrophotometer between the standard cell compartment and the photocell housing. Conventional 1-cm. cells can be used at any time by moving the multiple cell to the blank position. Figure 4 illustrates the cells and the complete assembly mounted in the spectrophotometer.

OPERATION

Cell Blanks. Because none of the multiple cells described has provision for accurate compensation for cell or solvent absorption, suitable blank corrections must be applied for accurate results. Blanks are required for each cell at each wave length used in making the measurements. The blanks are usually determined for gases with the cells evacuated, and for liquids with the cells filled with some of the solvent used in making the solutions. It is not usually necessary to redetermine the blanks for each sample; instead, one set of blanks usually suffices for a whole series of samples.

Gases. All of the multiple cells are intended for direct connection to a gas-handling system so that a gas sample can be introduced into the cell and the pressure adjusted to any desired value with the cell in the spectrophotometer. This is readily accomplished with the sector cells through the spherical joint connection on the end of the spindle. When this connection is properly lubricated, the sector can be rotated to any desirable position without causing any leaks. The liquid cell for the Beckman spectrophotometer requires a more complicated connection because of the sliding motion. Although this cell has not yet been used for gases, experience with a similar cell has shown that the required motion can be obtained by using two horizontal connecting lines and three pairs of male and female spherical joints. These joints are sealed to the ends of the tubes with their axes vertical to allow horizontal rotation of the two tubes around the joints.

Liquids. The procedures used for filling the two liquid cells differ only in minor details. In each case the sample is drawn through the series of cells by applying light suction to the exit side. A convenient arrangement is to connect the cell to a source of low vacuum through a glass T-tube. One end of this T-tube is connected to the vacuum, another end carries a female spherical joint to fit the exit connection to the cell, and the third side is left open. The suction through the cell is then controlled by placing a finger over the open side. The inlet connection for the Cary cell consists of a glass capillary tube with a female spherical joint sealed at right angles near the upper end. The sample is drawn into the cell by immersing the lower end of the capillary into the sample and applying suction. The liquid cell for the Beckman spectrophotometer is filled through a small funnel attached to the inlet side of the cell.

The cells are not normally cleaned and dried between samples but merely flushed out with the solution of the next sample. This is accomplished by first sucking out through the exit connection as much of the previous solution as possible. The cell is then filled with the new solution, which is in turn sucked out. In this manner the cells are flushed with three portions of the new sample and then refilled for the measurement. After the cell is filled, the vacuum connection is removed and a cap is placed on the spherical joint to retain the sample in the cell.

The above operation requires about 25 ml. of solution to fill the multiple cell for the Cary spectrophotometer and a little less for the Beckman. Smaller samples are handled by first cleaning and drying the cell and then drawing in the sample. This procedure requires only 6.3 and 3.0 ml. for the Cary and Beckman cells, respectively.

Measurement of Cell Thicknesses. The thickness of the individual cells in each of the three assemblies is conveniently determined by comparing the absorbance of an appropriate dilution of some substance in the cell with the absorbance of another dilution in a cell of known thickness (usually 1 cm.) and applying the Beer-Lambert law to the data. Diphenyl has been found to be satisfactory for this purpose. A solution of diphenyl in a paraffinic solvent shows a broad absorption maximum near 2470 A. with an absorptivity of approximately 109 liters per gram cm. The broad peak has the advantages of making the absorbance measurement less sensitive to small changes in the wave length or slit width. The high absorptivity has the advantage of allowing the use of dilute solutions, even in the thinnest cells. These dilute solutions minimize the possibility of deviations from Beer's law. Even if more concentrated solutions are used, it is probable that the major effect would be a small shift in the position of the absorption maximum rather than a change in the intensity at the peak. Such an effect probably would not be detectable in view of the broad absorption peak.

The thickness measurements of the liquid cells are quite straightforward. Several dilutions are prepared for each cell to give absorbances in the optimum range for accurate measurement. The data are corrected for both cell and solvent blanks. The measurements on the Beckman gas cell are somewhat inconvenient, in that this cell is not readily filled with a liquid. However, the filling can be accomplished by removing the sector from the compartment and unscrewing the small plug in the outer wall of the reservoir. The solution is then drawn into the cell by applying suction to this opening.

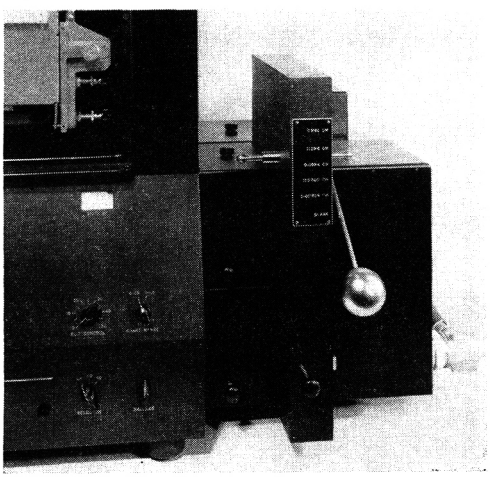
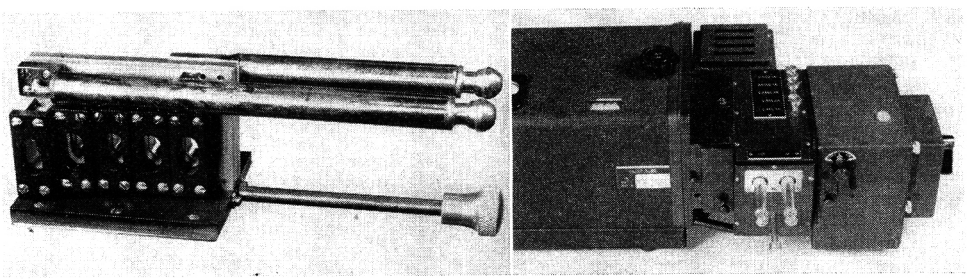


Figure 3. Multiple cell assembly for Cary spectrophotometer



Unmounted Mounted

Figure 4. Liquid cell assembly for Beckman DU spectrophotometer

APPLICATIONS

Analysis of Gases. Multiple cells are particularly desirable for measuring the absorbance of a gas when any of the following conditions apply: Accurate analyses are required for both high and low concentrations; the absorptivity varies widely over the spectral region of interest; the absorptivity is too high in a 1-cm. cell for accurate pressure measurement with an ordinary manometer.

Methods used in this laboratory for the determination of 1,3-butadiene in C_4 hydrocarbons and for the determination of isoprene, *cis*-piperylene, *trans*-piperylene, and cyclopentadiene in C_5 hydrocarbons illustrate each of these points. ASTM method D 1096-54 for the determination of 1,3-butadiene specifies measurements in a 1-cm. cell at 2410, 2350, 2300, or 2260 A. The longer wave lengths, where the absorptivity is lower, are used for analyzing samples with high butadiene contents and the shorter wave lengths for samples of low concentration. Unfortunately, measurements at the longer wave lengths are considerably influenced by the presence of small amounts of C_5 or higher dienes. The effect of such contaminants is minimized by basing the analysis on a measurement at 2160 A. and covering the required range of concentration by the use of different cell thicknesses. Measurements on high concentrations of butadiene in a regular 1-cm. cell are not very practical at this wave length, because the absorbance of pure butadiene would be 0.9 for a pressure of only 1 mm.

The determination of the C_5 dienes is based on measurements at 2570, 2290, 2240, and 2220 A. Because the absorptivities of the various pure dienes vary by a factor of nearly 200 through this spectral interval, this analysis would be difficult using a single cell thickness.

The prototype of the Beckman triple gas cell did not at first contain a reservoir. This led to some difficulties in the analysis of C_5 dienes. This particular arrangement contained a 1.00- and a 0.05-cm. cell connected in parallel. When the cells were filled to a low pressure with a synthetic mixture of a C_5 diene and isopentane, the absorbance observed for the 0.05-cm. cell was considerably less than the value calculated from measurements on the pure diene. The absorbance increased with time until in about 30 minutes it approached the calculated value. A similar but much smaller effect was observed for the 1.00-cm. cell. No such change in absorbance was observed for the pure diene under similar conditions. This difficulty completely disappeared when the two cells were connected to a 25-ml. reservoir arranged so that the sample first passed through the two cells in series and then into the reservoir. Although the cause of this phenomenon has not been established it is suspected to be due to fractionation resulting from a slight tendency of the diene molecules to adsorb preferentially on the walls of the connecting lines. Thus, the first small quantity of gas passing through a previously evacuated line would be depleted in diene content. Although neither of the two liquid cells has a built-in reservoir, this is readily added when necessary by connecting a small glass bulb to the exit side of the cell.

Analysis of Liquids. The first two conditions given above regarding the advantages of a multiple cell for the analysis of gases also apply to the analysis of liquids. The common procedure of analyzing solid or liquid samples by preparing a trial dilution, checking its absorbance, preparing a new dilution, and cleaning and refilling the cell usually can be avoided by using a multiple cell and a solution made up to some standard concentration. Even samples that can be diluted properly the first time sometimes may be analyzed more efficiently with a multiple cell because of the ease of filling the cell and because of the possibility of analyzing a more concentrated solution. The multiple cell is least efficient for the analysis of a single sample because of the necessity of determining cell blanks. The availability of thin cells is also an advantage for measurements near 2000 A. because of the difficulty in purifying solvents so that they are adequately transparent for use in a 1-cm. cell.

Multiple cells also have been found useful for making measurements at fixed wave lengths with the Cary spectrophotometer. In this case the absorbance is determined by setting the spectrophotometer to each of the required wave lengths in turn, and then rotating the cell until the absorbance falls in the desired range. It is usually more convenient to read the absorbance directly from the pointer and scale above the recorder pen than to record the reading on the chart paper.

Recording Spectra. The usual practice for recording a complete spectrum in this laboratory with the multiple cell is to start with the thickest cell at the longest wave length and then record toward shorter wave lengths until the absorbance becomes too high. The wave length and chart paper are then reset to a slightly longer wave length to give some overlap and the recording is resumed with a thinner cell. This procedure is continued until the complete spectrum has been obtained through the desired spectral region. Occasionally it is necessary to prepare a second dilution and refill the cell in order to obtain the complete spectrum.

Cell and solvent blanks must be applied whenever accurate absorptivities are required. However, conclusions regarding the sample are often based on the shape of the spectrum, the location of the absorption peaks, and on the absorptivities at a few selected wave lengths. Correcting for blanks at these few wave lengths is not much of a problem. Often corrections can be completely avoided by the use of base-line techniques.

Measurements on Stream Samples. Multiple cells should be useful for following changes in the absorbance of flowing streams. The 100-fold range of thickness available greatly facilitates such measurements both by having the required cell thicknesses immediately available and by making a cell thickness change possible during the experiment. One possible application of this type occurs in the study of separation processes such as chromatography. Another possible application is following the progress of a chemical reaction by continuously circulating a small stream from the reaction vessel through the cell.

CONTEMPLATED IMPROVEMENTS

The principal disadvantage of the multiple cell is the necessity of applying cell and solvent blanks to obtain accurate absorptivities. The windows on the Cary cell were originally carefully selected so that the absorbance of each cell, when filled with a transparent solvent, closely matched the absorbance of the quartz reference plate. Unfortunately, the cell blanks slowly increase with use. A periodic cleaning with a chromic–sulfuric acid mixture improves the blank value but does not return it to the original value. The thin white deposits that slowly form on the windows may be lead sulfate from action of sulfuric acid on the lead gaskets. In addition, the stainless steel and the amalgamated lead gaskets now employed probably would greatly handicap the application of the present multiple cells to any colorimetric method for determining metals because of the danger of contamination. Consequently, it might be worth while to modify the construction so that the sample would contact only inert materials such as quartz, Teflon, or gold. Although details have not been worked out, this type of construction appears to be feasible without an excessive increase in cost.

ACKNOWLEDGMENT

The author wishes to acknowledge the help and cooperation of B. M. Burchard of these laboratories and of personnel at Beckman Instruments and the Applied Physics Corp. in the design and construction of the cells.

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RECEIVED for review June 11, 1956. Accepted August 16, 1956.

Spectrophotometric Titration of Spinal Fluid Calcium and Magnesium

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A simple procedure for the spectrophotometric titration of calcium and magnesium in cerebrospinal fluid involves the separate determination of the individual cations of the same sample instead of the use of the difference between total divalent cation and either constituent. The several phases of the titration have been investigated: dye variance, constitution of the titrant, spectral studies, and accuracy and precision of the determination.

CALCIUM and magnesium, two of the more commonly determined metals, have been quantitatively determined by a number of comparatively recent complexometric procedures. Some of these have been concerned with visual titrimetry involving the use of the indicators murexide, Eriochrome black T, or phthalein complexon (*1, 2, 4, 10, 12, 18, 21*), while several spectrophotometric titrations employing the same indicators for either one of the two cations or total cations appear to have been successfully investigated (*3, 6–8, 13–15, 19*). The employment of individual spectrophotometric titrimetry for small volumes (1 to 2 ml.) of low concentration mixtures of calcium and magnesium within the fraction of a milliequivalent range (0.001 to 0.005 meq. per sample analyzed) for both metals incorporated within the same medium has seen but little investigation. This is the circumstance when a physiological medium such as spinal fluid is the material being examined.

Most spectrophotometric titrations appear to have been involved with the determination of serum calcium, using murexide as the indicator. Visual titrations, however, have been much more commonly employed. Sobel and Medoff (*20*) developed a visual microtechnique for calcium and magnesium by titrating the total divalent cations present using Eriochrome black T, then adding oxalic acid and excess nickel ions, and titrating the liberated magnesium and residual nickel to get the calcium value. The magnesium was then calculated by difference. Friedman and Rubin (*9*), using a modification of Diehl's procedure (*5*), visually titrated the total divalent cations of serum and spinal fluid, using Eriochrome black T as the indicator. Magnesium was then determined in the supernatant fluid of a second sample after calcium had been precipitated as the oxalate.

The difficulty involved in objectively seeing the end point change, when an aliquot of a solution of but several milliequivalents per liter is used, can be overcome by spectrophotometric substitution for the visual means of quantitation (*7, 16, 19*). When the very dilute titrant required to get a satisfactory titer is employed, 0.001*M* or less, the end point creeps up and usually appears too early.

The work reported here represents a study of the phases of the titration as well as a presentation of a relatively simple procedure for determining calcium and magnesium in spinal fluid, where each component from the same starting sample is analyzed individually.

REAGENTS

All chemicals were of reagent grade, unless otherwise specified.

Calcium Carbonate Standard Solution. Weigh out 250 mg. of dried analytical reagent grade calcium carbonate, transfer to a 1-liter volumetric flask containing about 300 ml. of distilled water, and dissolve by adding a minimum amount of concentrated hydrochloric acid. Dilute to the mark of the flask with mixing.

Magnesium Stock Standard Solution. Weigh out 1 gram of pure magnesium and dissolve in distilled water with the addition of a minimum amount of concentrated hydrochloric acid. Dilute to the mark of a 1-liter volumetric flask with mixing.

Magnesium Working Standard Solution. Pipet 5 ml. of the stock standard into a 100-ml. volumetric flask and dilute to the mark with mixing.

Buffer Solution, Precipitating. Weigh out 1.5 grams of ammonium oxalate and dissolve in a 250-ml. volumetric flask containing about 200 ml. of distilled water. Add 0.35 ml. of concentrated ammonium hydroxide and 3.5 grams of ammonium chloride. Dilute to the mark of the flask and mix well.

Buffer Solution, Titrating (5). Weigh out 67.5 grams of ammonium chloride and dissolve in a 1-liter volumetric flask containing 2 to 300 ml. of distilled water. Add 570 ml. of concentrated ammonium hydroxide, dilute to the mark of the flask with mixing, and store in the refrigerator.

Ethylenediaminetetraacetic Acid Titrant (EDTA). Weigh out 400 mg. of dried analytical reagent grade ethylenediaminetetraacetic acid [(ethylenedinitrilo)tetraacetic acid, EDTA] and transfer to a 1-liter volumetric flask containing several hundred milliliters of distilled water with four sodium hydroxide pellets dissolved in it. Add 10 ml. of the magnesium stock solution and dilute to the mark of the flask with mixing after the ethylenediaminetetraacetic acid has gone into solution.

Eriochrome Black T Indicator. Weigh out 50 mg. of Eriochrome black T dye, dissolve in distilled water, and dilute to the mark of a 50-ml. volumetric flask. This dye is satisfactory for the titration for 2 weeks, when it is kept refrigerated.

Ammonium hydroxide, 2%.

Perchloric acid, 72%.

APPARATUS

Coleman, Jr., spectrophotometer, Model 6A.

Cuvettes, 19 by 150 mm., or borosilicate glass test tubes of the same size.

PROCEDURE

Standardization of Calcium. Pipet 1.0 ml. of the calcium standard solution into a cuvette and add 1.0 ml. of distilled water and 2.0 ml. of the precipitating buffer solution. Mix well and allow to stand for 1 hour. Centrifuge at 3000 r.p.m. for 15 minutes and carefully decant the supernatant fluid. Add 4.0 ml. of the 2% ammonium hydroxide solution, mix well, and recentrifuge at 3000 r.p.m. for 15 minutes. Decant the supernate and drain. Add 0.05 ml. of perchloric acid and put in a hot sand bath for 10 minutes. The tube fills with dense fumes of perchloric acid during this period of time. The temperature is approximately in the vicinity of 200° C. Cool, and add 3.0 ml. of water, 3.0 ml. of the titrating buffer, and 0.2 ml. of Eriochrome black T. Set the instrument to read 0.5 to 0.6 at 660 mμ and titrate in the spectrophotometer, mixing well after the addition of each increment and then recording the absorbances until the upper plateau range has been reached.

The intersection of the steeply ascending portion of the curve and the upper plateau represents the end point. No blank is used in the titration, because dilution does not affect the end point. During the short period involved in the titration no drift was caused by instability of the indicator, which is fairly stable, or the battery-operated spectrophotometer.

Standardization of Magnesium. Pipet 1.0 ml. of the magnesium working standard into a test tube and add 1.0 ml. of distilled water and 2.0 ml. of the precipitating buffer solution. Mix well and then pipet a 3.0-ml. aliquot into a cuvette or test tube. Add 3.0 ml. of the titrating buffer and 0.2 ml. of Eriochrome black T solution, set the instrument at 0.05 to 0.10 with the sample at 660 mμ, and titrate as for calcium. Again, no blank is required, as the sample acts as its own blank.

Cerebrospinal Fluid Analysis. Pipet 2.0 ml. of spinal fluid into a cuvette, add 2.0 ml. of precipitating buffer, and allow the precipitate of calcium oxalate to form for 1 hour. Centrifuge at 3000 r.p.m. and carefully decant the supernatant fluid into a clean container. Pipet 3.0 ml. of the supernate into a cuvette, add 3.0 ml. of the titrating buffer and 0.2 ml. of dye, and titrate

as for the magnesium standard. Wash the precipitate in the first cuvette with 2% ammonium hydroxide and proceed as for the calcium standard.

DISCUSSION

Previous work appearing in the literature has shown that the end points in a spectrophotometric titration of calcium or magnesium, when EDTA is used as the titrant along with metal specific indicators, are sharper and more accurate at the wave length where the absorbance difference is greatest between the metal-containing indicator form and the metal-free indicator form (*8*, *13*).

Figure 1 shows the spectral curves in the visible range for several of the media encountered in magnesium and calcium determination—i.e., water, spinal fluid, and serum. The water solution used contains calcium, magnesium, ammonium phosphate, and sodium and potassium chlorides in the physiological range expected. Absorbance maxima were obtained on the one hand by the addition of a fixed amount of dye to the buffered medium and on the other hand by the addition of a small amount of solid EDTA to the same system, to remove the divalent cations from the dye complexes. In the work of Karsten and associates (*13*) the wave length of 530 mμ was chosen because it best suited their macro system and their instrumentation. But in view of the fact that a greater change in absorbance is obtained beyond 600 mμ, it was felt that a greater sensitivity would enable a more effective titration when small amounts of calcium and magnesium are involved. The spectral curves are not quite the same for the different media shown here, although water and spinal fluid, as might be expected, resemble each other closely.

As Eriochrome black T works well with magnesium and very poorly with calcium (*5*, *17*), it was necessary to incorporate magnesium into the EDTA titrant. The amount of magnesium added was varied from 0 to 30 γ per milliliter of titrant, whereas the uncomplexed EDTA was held fairly constant at 400 mg. per liter. Curve *A* of Figure 2 shows the poor data plot graphed for a calcium titration with no magnesium present, while *B* to *E* show the curves obtained for 2, 7.5, 10, and 30 γ per milliliter of titrants, respectively. A standard solution containing 10 γ of magnesium and 0.4 mg. of EDTA per milliliter was decided upon as most suitable for the titration. This corresponded roughly to about a 0.001*M* solution of uncomplexed EDTA.

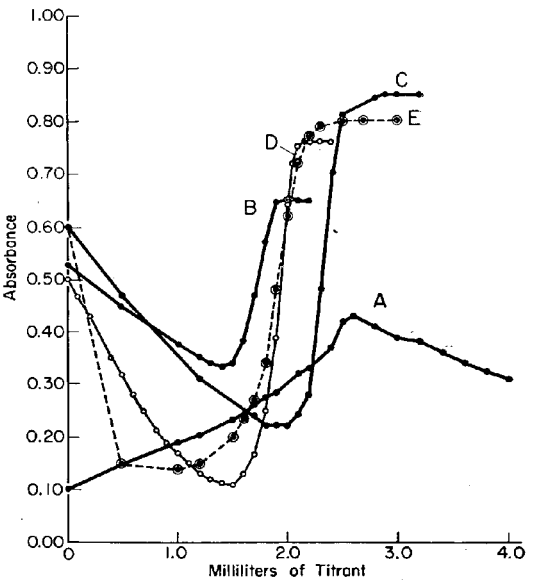

Figure 2. Effect of varying magnesium concentration of EDTA in calcium titrations

A. No magnesium
B. 2 γ per ml.
C. 7.5 γ per ml.
D. 10 γ per ml.
E. 30 γ per ml.

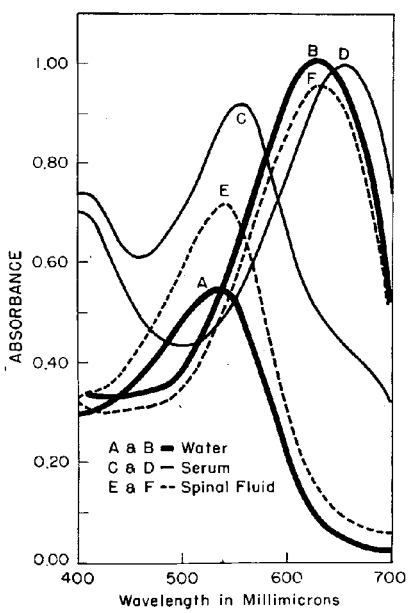

Figure 1. Spectral curves in the visible range for metal-containing and metal-free complexes

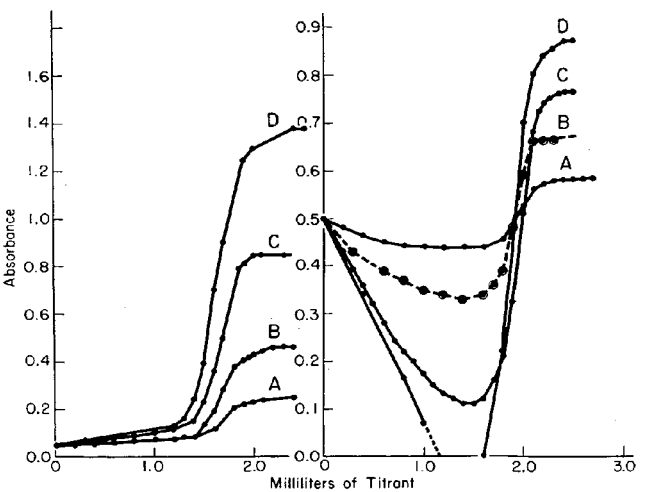

Figure 3. Effect of variation in dye concentration for magnesium (*left*) and calcium (*right*)

A. 50 γ
B. 100 γ
C. 200 γ
D. 300 γ

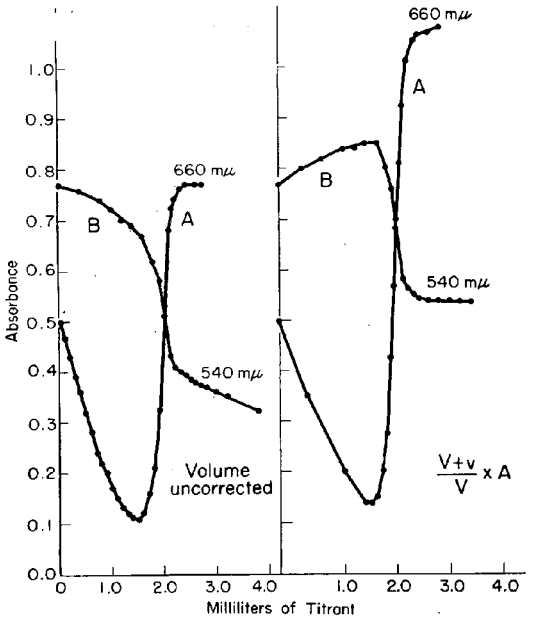

Figure 4. Titration curves for calcium at 540 and 660 mμ uncorrected (*left*) and corrected (*right*) for dilution with titrant

Table I. Determination of Absolute Amounts of Calcium and Magnesium in Their Mixtures

(Milliequivalents per liter)

Calcium Present	Calcium Found	Magnesium Present	Magnesium Found
1.25	1.27	2.10	2.12
2.50	2.49	2.10	2.10
3.75	3.65	2.10	2.10
5.00	4.95	2.10	2.10
2.50	2.54	1.05	1.17
1.25	1.23	2.10	2.12
2.50	2.48	3.15	3.14
2.50	2.40	4.20	4.18
2.50	2.42	2.10	2.02
3.75	3.58	3.15	3.28

Table II. Determination of Absolute Amounts of Calcium and Magnesium in Mixtures in the Presence of Normal Amounts of Sodium, Potassium, and Phosphate

(Milliequivalents per liter)

Calcium Present	Calcium Found	Magnesium Present	Magnesium Found
1.25	1.28	2.10	2.15
2.50	2.46	2.10	2.10
3.75	3.77	2.10	2.13
5.00	4.98	2.10	2.15
2.50	2.46	1.05	1.10
2.50	2.48	2.10	2.13
2.50	2.56	3.15	3.50
2.50	2.48	4.20	4.42
2.50	2.50	2.10	2.20
3.75	3.67	3.15	3.08

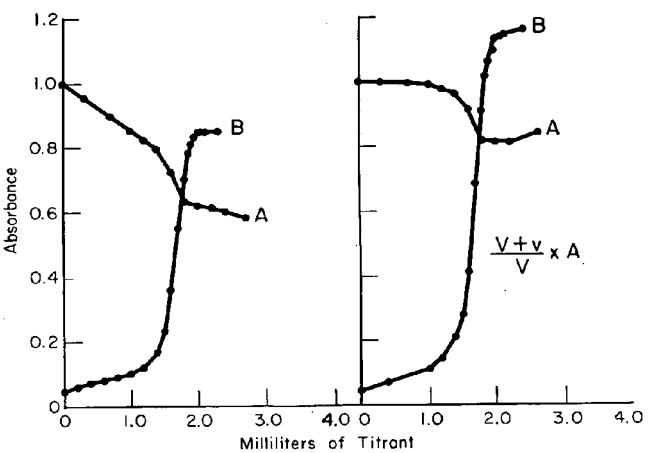

Figure 5. Titration curves for magnesium at 520 and 660 mμ uncorrected (*left*) and corrected (*right*) for dilution with titrant

In a calcium titration, the sharp drop in absorbance with the early additions of standard solution indicates the exchanging of magnesium in the EDTA complex with the free calcium left untitrated. As not enough dye is added to complex the total amount of cation being titrated, the initial portion of the titration does not show a change in absorbance due to the cation being released from the dye-cation complex, and this phenomenon is not demonstrated until the ascending portion of the titration is reached. The steep climb to the metal-free complex form of the dye takes place with small increments of titrant, to the upper plateau where added titrant merely involves dilution of the system. The intersection of the rising portion of the curve with the upper plateau represents the end point of the titration. Visually, it is difficult to attain this intersection and the end point appears early, in the ascending part of the titration. This is especially true for dilute titrant and where the milliequivalents of divalent cation determined are in the cerebrospinal fluid range.

One of the criteria for accuracy in this type of titration is the steepness of the ascending portion of the curve, and this is partially dependent on the dye concentration of the system. Fortuin, Karsten, and Kies have demonstrated (*8*) that increasing this concentration for a much higher cation range than is shown here in this investigation yields deviations from the desired vertical to a point where the lower plateau disappears and the ascent begins at the same instant as the titration. The slope of the ascending portion of the curve is therefore inclined more toward the horizontal. The effect of varying the amount of dye present in the system was then investigated for both calcium and magnesium. Figure 3 shows the data graphed for magnesium and calcium, respectively, for titrations in which the dye concentration was varied from too little dye (too small a change in absorbance between the metal-free indicator form and the metal-containing indicator form) to too much dye (too high an absorbance for the upper plateau). The amounts used were 50, 100, 200, and 300 γ of Eriochrome black T. Inspection of the data showed that 200 γ of dye gave a satisfactory plot for calculation—curves *C* for both metals involved. If a little more or a little less dye were added, it would still not change the end point determination.

The spectral curves of Figure 1 indicate that two areas show

differences between the two dye forms, one in the 600- to 700-mμ range, and the other in the 480- to 560-mμ range. It was decided to compare the two areas to see if both were favorable. Figure 4 shows the curves obtained for calcium at 540 and 660 mμ and Figure 5 shows the curves for magnesium at 520 and 660 mμ. The absorbance break is much steeper at 660 mμ. When all curves were corrected for the dilution of absorbance by the titrating solution (*11*), those obtained at 520 and 540 mμ, still did not exhibit very large absorbance differences for the two dye forms, while the 660-mμ curves were increased by a much larger factor. However, the end points at 660 mμ for dilution-corrected or -uncorrected data are easy to plot and give the same results, so that for the purpose of titrating in the low milliequivalent range of normal and pathological spinal fluids 520 or 540 mμ appear to be somewhat less desirable.

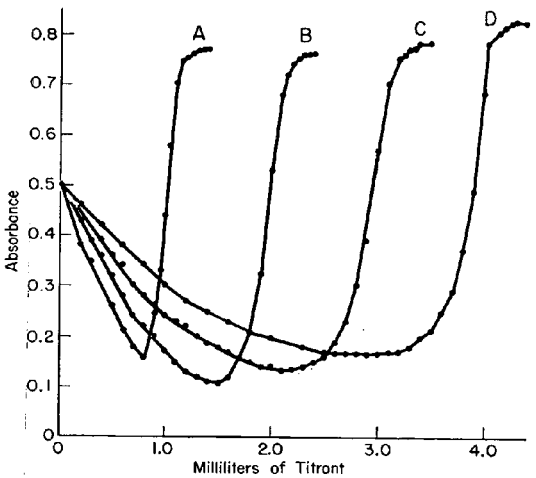

Figure 6. Titrimetric curves for calcium at 660 mμ

Meq. per liter of spinal fluid
A. 1.25
B. 2.50
C. 3.75
D. 5.00

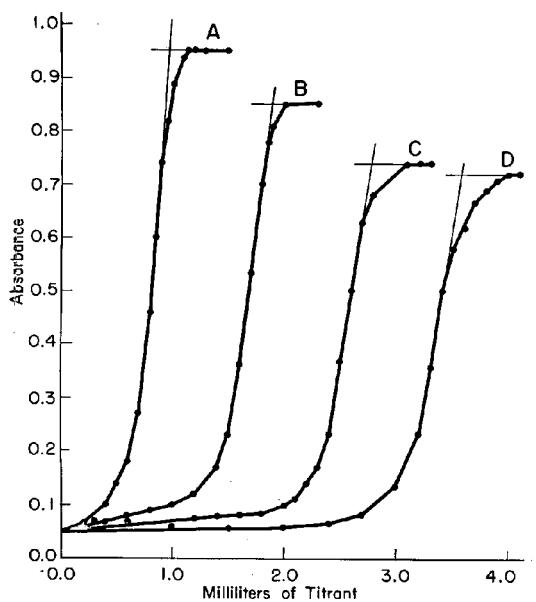

Figure 7. Titrimetric curves for magnesium at 660 mμ

Meq. per liter of spinal fluid
A. 1.05
B. 2.10
C. 3.15
D. 4.20

Recovery studies were then carried out to test the accuracy of the procedural techniques involved. These include four phases of investigation.

1. Mixtures of calcium and magnesium alone were analyzed according to the procedure described. For several samples, the calcium content was kept constant while the magnesium content was varied, and then the reverse procedure was followed (Table I). The analytical accuracy appears to be excellent.
2. A second set of mixtures of pure calcium and magnesium solutions contained both cations in the same proportions as in the experiments reported in Table I, but with normal amounts of sodium, potassium, and phosphate. Recoveries were made and the results are shown in Table II. The accuracy was not decreased by addition of these constituents, which are always present in cerebrospinal fluid.
3. A third set of mixtures was prepared, similar to those described in Table II, but to these were added comparatively large amounts of proteins in the form of human albumin and gamma-globulin, obtained through the Red Cross. The recoveries made are shown in Table III.
4. A number of pooled spinal fluids were tested and to each separately was added a fixed amount of calcium and then magnesium. Each sample was analyzed three times—once for calcium and magnesium to which no addition had been made, a second time for calcium after calcium had been added, and a third time

Table III. Determination of Absolute Amounts of Calcium and Magnesium in Mixtures with Sodium, Potassium, Phosphate, and Protein[a]

(Milliequivalents per liter)

Calcium Present	Calcium Found	Magnesium Present	Magnesium Found
1.25	1.25	2.10	2.14
2.50	2.48	2.10	2.05
3.75	3.79	2.10	2.02
5.00	5.00	2.10	2.07
2.50	2.44	1.05	1.05
2.50	2.51	2.10	2.10
2.50	2.44	3.15	3.24
2.50	2.51	4.20	4.18
2.50	2.44	2.10	2.10
3.75	3.80	3.15	3.25

[a] 40 mg. of albumin and 80 mg. of gamma globulin obtained through the Red Cross were dissolved in the solution.

Table IV. Recoveries of Additions of Calcium and Magnesium Made to Pooled Spinal Fluid[a]

(Milliequivalents per liter)

Sample	Calcium Present	Calcium Added	Calcium Found	Magnesium Present	Magnesium Added	Magnesium Found
A	3.61	..	..	2.20	..	..
A	..	1.25	5.00	..	..	..
A	..	..	..	..	2.10	4.20
B	2.51	..	..	3.68	..	..
B	..	2.50	5.22	..	..	..
B	..	..	..	..	1.05	4.63
C	2.52	..	..	2.50	..	..
C	..	2.50	4.70	..	..	..
C	..	..	..	..	2.10	4.54
D	2.61	..	..	2.59	..	..
D	..	1.25	3.85	..	..	..
D	..	..	..	..	1.05	3.60
E	2.42	..	..	2.72	..	..
E	..	2.50	5.10	..	..	..
E	..	..	..	..	1.05	3.65

[a] Random cerebrospinal fluid samples taken from clinical laboratories.

for magnesium after magnesium had been added. In other words, all analyses were made but once, but in effect each mixture was analyzed in triplicate. These results are shown in Table IV.

With the exception of an occasional determination, the analytical results for the recoveries using calcium or magnesium appear to be equally good, regardless of whether pure calcium and magnesium mixtures alone or other mixtures were analyzed.

Figure 6 shows the titrimetric curves obtained at 660 mμ for varying concentrations of calcium between 1.25 and 5.0 meq. per liter of spinal fluid. The intersection of the tangent to the steep ascending slope of the titration curve to a line parallel to the abscissa and tangent to the upper portion of the curve indicates the end point.

A similar study is shown in Figure 7, where increasing concentrations of concentration of magnesium in the range of 1.05 to 4.20 meq. per liter of spinal fluid are titrated at 660 mμ. The end point is graphically determined in the same manner as for calcium.

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RECEIVED for review January 7, 1956. Accepted July 18, 1956. Division of Biological Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955. Supported in part by a Grant-in-Aid from the Receiving Hospital Research Corp.

Photometric Determination of Chlorides in Water

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A method has been devised for the photometric determination of small amounts of chloride in water. The method is based on the displacement of thiocyanate from mercuric thiocyanate by chloride ion and the subsequent reaction of the liberated thiocyanate with ferric iron to form the colored complex $[Fe(SCN)]^{++}$, which is measured either visually or in a spectrophotometer. Concentrations of chloride as low as 0.05 p.p.m. can be determined.

THE literature on the determination of chlorides is voluminous. Whether present as a required constituent or as an impurity, the chloride ion is usually determined by either gravimetric or volumetric methods. The oldest and the classical method is the gravimetric, in which the chloride ion is evaluated as silver chloride. Another method frequently employed is the volumetric, several variations of which are available. The Volhard method, originated by Carpentier (*7*), described by Volhard (*38*), and later improved by Lundbak (*25*) and others (*26*), is more accurate than the Mohr method (*27*).

A comparatively recent method is the mercurometric method, which was developed in 1933 by Dubský and Trtílek (*9, 10*). Diphenylcarbohydrazide was used as an indicator in the titration with mercuric nitrate. Other workers (*1, 5, 8, 20, 22, 29, 32, 34*) later adopted this method with some modifications. All these methods, however, are not always suitable for the determination of micro quantities of chloride.

The present investigation was the result of a need for a simple colorimetric method for the determination of less than microgram quantities of chloride in condensate. Luce, Denice, and Akerlund (*24*) determined small amounts of chloride turbidimetrically. This method, however, lacked the required precision. Other methods (*2, 4, 6, 14, 15, 17, 21, 28, 35, 39*) for the determination of small amounts of chloride either required special apparatus or lacked the desired simplicity.

The method presented here is a modification of that proposed by Utsumi (*36, 37*) and followed up by Iwasaki (*16*). This modified procedure has been greatly improved and broadened in its application. The use of ferric perchlorate instead of ferric ammonium sulfate eliminates a variable inherent in the latter reagent. Use of an aqueous instead of an alcoholic solution of mercuric thiocyanate minimizes the glaring blank when visual color comparisons are made. The improved sensitivity thus obtained and the adaptability to either visual or spectrophotometric comparison made it useful for a more varied application. Although designed for the determination of chloride in condensate, it can also be applied in other fields.

REAGENTS

Mercuric thiocyanate, saturated water solution (0.07%).

Ferric perchlorate. Dissolve 6 grams in 100 ml. of 4*N* perchloric acid. This reagent may also be prepared by dissolving 14.0 grams of pure iron wire in dilute nitric acid. Upon dissolution of the iron, add 120 ml. of perchloric acid and heat the solution until it fumes. Continue heating until the solution turns purple, then cool and dilute to 1 liter.

PROCEDURE

Place 10 ml. of sample in a 50-ml. volumetric flask, add 5 ml. of 60% perchloric acid, 1 ml. of mercuric thiocyanate, then 2 ml. of ferric perchlorate. Make up to volume and mix well. Allow to stand for 10 minutes, then read the transmittancy on a spectrophotometer at 460 mμ. The color can also be compared visually with suitably prepared standards.

Preparation of Standards. Weigh 1 gram of dried C.P. sodium chloride to the nearest milligram. Dissolve in distilled water, transfer to a 1-liter volumetric flask, and dilute to volume. Each milliliter contains 1 mg. of sodium chloride. Pipet 10 ml. of this solution into a 1-liter volumetric flask and dilute to volume. This master solution, containing 10 p.p.m. of sodium chloride, is used to prepare standards containing chloride concentrations from 0.05 to 5 p.p.m.

DISCUSSION

The existence of a distinctive color reaction between Fe^{+++} and SCN^- ions has been known for over a century. This reaction has been employed in the colorimetric determination of iron.

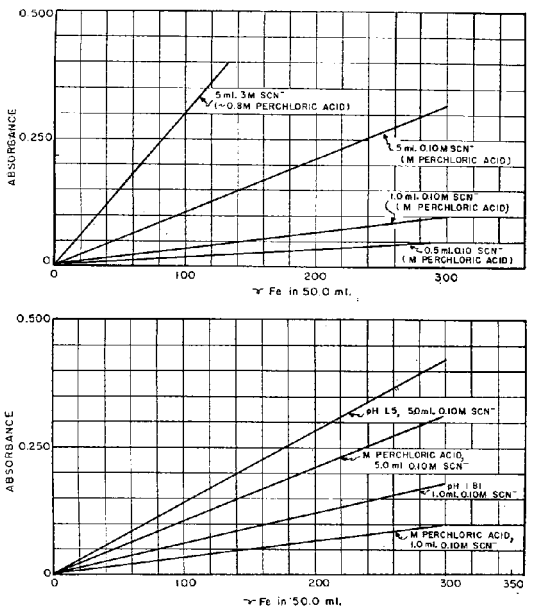

Figure 1. Influence of various thiocyanate–perchloric acid concentrations on absorbance

The first investigation into the aspects of the iron-thiocyanate reaction appeared in 1931 (*33*), when Schlesinger and Van Valkenburgh (*33*) made a spectral study of the iron-thiocyanate complex. They measured the light absorption of aqueous solutions of ferric thiocyanate and $Na_3[Fe(SCN)_6]$, and of anhydrous ether solutions of ferric thiocyanate. They concluded from the similar spectra obtained that the same absorbing species is probably responsible for the color in every instance. Kiss, Abrahám, and Hegedüs (*18*) confirmed this work and concluded that, with excess thiocyanate ion, the principal absorbing species is $[Fe(SCN)_6]^{---}$

Bent and French (*3*) and Edmonds and Birnbaum (*12*) proved the existence of $FeSCN^{++}$ in ferric thiocyanate solutions. They presented evidence that when ferric iron is in excess the iron thiocyanate is present as $FeSCN^{++}$ and is the only absorbing species.

On the other hand, Frank and Oswalt (*13*) showed that, at total concentrations greater than about 0.004*M*, an excess of thiocyanate leads to a higher absorbance than the same excess of ferric iron. Thus, when ferric iron is in excess there is only one equilibrium involved: the one leading to the formation of $FeSCN^{++}$. When thiocyanate is in excess the appearance of $Fe(SCN)_2^+$ becomes evident (*Figure 1*).

In addition to the two complexes indicated, others are possible when thiocyanate reacts with iron. A series of complexes, represented by $Fe(SCN)_n^{+3-n}$, where $n = 1, \ldots 6$, can be obtained (*19*). Equilibria data for six complexes formed by the interaction of iron and thiocyanate are given by Lewin and Wagner (*23*).

All these complexes are red. There is not much difference in hue, although there is a shift in maximum absorbance with various acid concentrations. In perchloric acid medium the maximum absorbance is obtained at 460 mμ (Figure 2). A shift to 495 mμ is obtained when sulfuric acid is used instead of perchloric acid. The addition of acetone does not affect the position of the peak materially.

Results of the determination of ferric iron in perchloric acid with excess thiocyanate are summarized in Figure 1.

Fifty per cent acetone solutions were much more stable than water solutions. The per cent transmittance of 50% acetone solution containing 306 γ of ferric iron had changed in 1 hour from 13.2 to 13.8%, introducing an error of less than 5%. Solutions containing nitric acid darkened on standing.

The visible absorption spectra of sulfuric acid–nitric acid solutions with and without acetone are markedly similar, with absorption maxima at 490 mμ. The maximum absorption spectrum of a solution of ferric iron with excess thiocyanate in 1*M*

Table I. Effect of Perchloric Acid Concentrations

(2 ml. of 0.25*M* ferric iron and 294 γ of thiocyanate in 50-ml. final volume)

Ml. 60% Perchloric Acid	Absorbance[a]
1.0	0.2622
2.0	0.2512
3.0	0.2440
4.0	0.2408
5.0	0.2406
6.0	0.2401

[a] 1-cm. cell.

Table II. Visual Estimation of Chlorides

(All values are in parts per million)

Concn. of Standard Chloride Solution	Concn. Found[a]
0.0	0.2
0.05	0.2
0.1	0.2+
0.2	0.3
0.3	0.4
0.5	0.5

[a] Identical results were obtained from 3 operators.

Table III. Influence of Other Ions on Chloride Determination

Contaminant	Amount Taken, Mg.	Chloride, γ Taken	Chloride, γ Found
F^-	0.0125	125	125
HPO_4^{--}	2.5	125	118
HPO_4^{--}	5.0	125	105
HPO_4^{--}	12.5	125	74
$NaNO_3$	0.05	40	39.5
	Grams		
$NaNO_3$	1.25	125	75
$(NH_4)_2SO_4$	0.1	25	15
$(NH_4)_2SO_4$	0.5	125	14
$(NH_4)_2SO_4$	1.25	125	0
Na_2SO_4	0.1	25	23
Na_2SO_4	0.5	125	12
Na_2SO_4	1.25	125	5

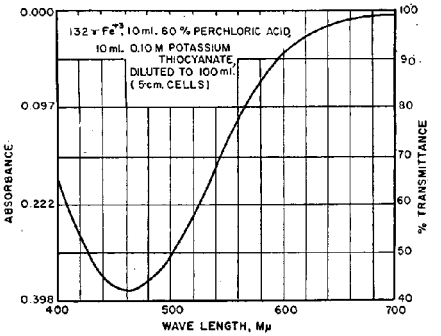

Figure 2. Absorption spectrum in perchloric acid

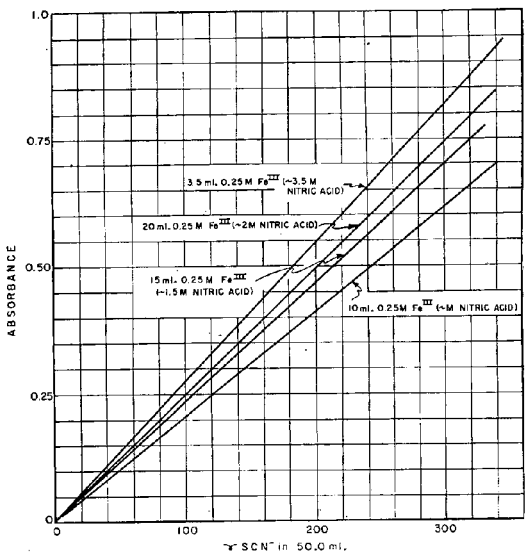

Figure 3. Effect of varying iron concentration

perchloric acid is at 460 mμ. It shows relatively less absorption at 400 mμ than the same solutions containing an equivalent amount of sulfuric acid.

The color intensity of the iron thiocyanate complex depends upon several variables: excess of thiocyanate (*18*), kind of acid, and time of exposure. In the application of this reaction to the determination of chlorides, the thiocyanate ion is measured and no excess is possible. The iron is in excess and its effect on the iron-thiocyanate complex was studied. Measured quantities of thiocyanate were allowed to react with various amounts of excess iron. Results of these experiments are shown in Figure 3. Acetone, having a low dielectric constant, intensifies the color (Figure 4).

The visible absorption spectrum of thiocyanate–excess ferric iron shows a pronounced absorption band between 400 and 430 mμ which the thiocyanate–excess ferric iron–acetone combination lacks. The absorption maximum for both solutions is at about 480 mμ.

Figures 4 and 5 show the effect of various acid concentrations on the colored complex. Ordinarily, the acidity of the solution does not affect the color intensity materially, provided the acid does not form a complex with the ferric iron. For instance, when nitric acid is used for acidification, the color intensity shows little change in the range from 0.05- to 0.08*N* (*31*). However, when larger concentrations of nitric acid are used, the color intensity changes somewhat with the acid concentration.

Table I shows the effect of perchloric acid concentrations. Large excess of acid offers no interference; however, the acidity cannot be decreased below a certain lower limit. Erratic results are obtained with low acid concentrations. Less concentrated perchloric acid can be tolerated when spectrophotometric measurements are made than when visual comparisons are made.

CHLORIDE DETERMINATIONS

Visual (Table II) and spectrophotometric determinations were made on prepared samples of known chloride content. The absorption spectrum without additional perchloric acid shows a secondary peak at 420 mμ (Figure 6) due to low acid concentration. In Figure 5 curve 2 is the most suitable for visual comparison, because a low blank value is evident. The system (Figure 5) does not follow Beer's law. However, up to 40 p.p.m. of chloride ion, a straight line is obtained and the intensity (chloride concentration) is proportional to the thiocyanate concentration. For the photometric evaluation, curve 3, Figure 5, is most sensitive, even though the blank is rather high.

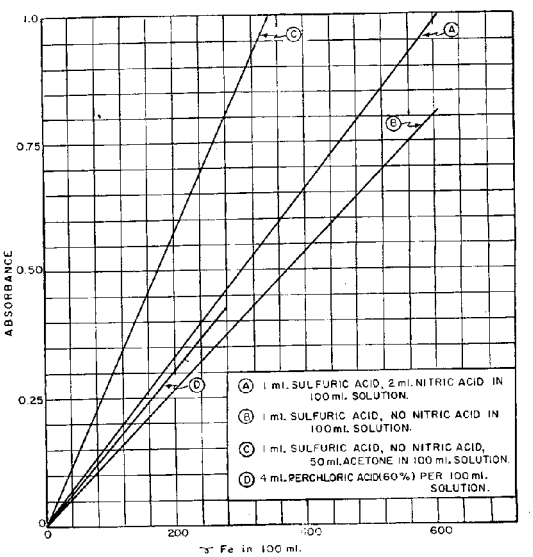

Figure 4. Calibration curves for iron in various acid media and influence of acetone

INTERFERENCES

The bromide ion interferes in any quantity. It displaces thiocyanate from mercuric thiocyanate in the same manner as the chloride ion does. Small amounts of fluorides, nitrates, nitrites, sulfates, and phosphates do not interfere. Higher concentrations of sulfates and phosphates bleach the color. High concentrations of ethyl and isopropyl alcohols, tartaric acid, and acetone impart a yellowish brown to the ferric thiocyanate color. The

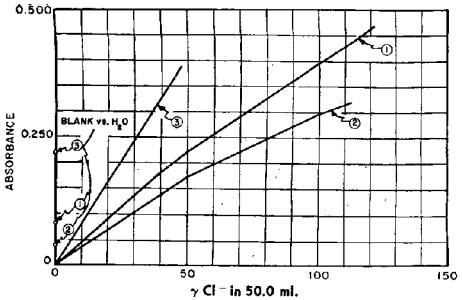

Figure 5. Effect of acid concentration on absorbance

2 ml. 0.25*M* Fe^{+++} in perchloric acid; 5 ml. saturated aqueous mercuric thiocyanate
1. pH 1.7
2. 1*M* perchloric acid
3. 1.5*M* perchloric acid (approx.)

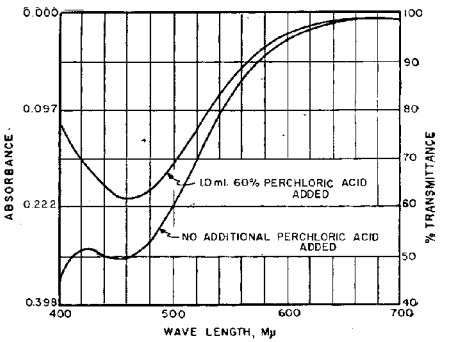

Figure 6. Absorption spectra of iron-thiocyanate complex in strong and weak acid media

80 γ chloride, 4 ml. of 0.25*M* Fe^{+++} in perchloric acid, 10 ml. saturated aqueous mercuric thiocyanate; final volume, 100 ml.; 5-cm. cells

blanks are also colored yellowish brown. Spectrophotometric data are summarized in Table III.

EXTRACTION

The iron-thiocyanate complex in the presence of excess iron is not extracted by ether or other organic solvents. It was pointed out by Durand and Bailey (*11*) in 1923 that the red color of the iron-thiocyanate complex is not extractable unless there is an excess of thiocyanate. This was confirmed by Peters and French (*30*). Molecular weight determinations by Schlesinger and Van Valkenburgh (*33*) in anhydrous ether and benzene showed the formula to be $Fe_2(SCN)_6$.

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RECEIVED for review October 5, 1955. Accepted April 6, 1956. Division of Water, Sewage, and Sanitation Chemistry, Symposium on Analytical Methods for Water and Waste Water, 130th meeting, ACS, Atlantic City, September 1956.

Polarographic Analyses of Mixtures of Prednisone and Cortisone

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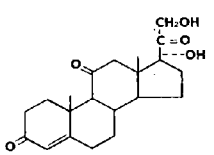
A polarographic procedure has been developed for the analysis of prednisone-cortisone mixtures with an accuracy within 4%. An additional procedure for use with mixtures rich (90% or over) in one component gives results good to 1%.

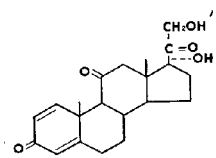
THE possibility of using the polarograph as an analytical tool in the analysis of Δ^4-3-ketosteroids was first pointed out by Eisenbrand and Picher (*1*). These authors examined testosterone, progesterone, and deoxycorticosterone in 90% ethanol solutions 0.1*N* in lithium chloride, and found a linear relationship between concentration and wave height for the compounds. Shortly thereafter, Wolfe, Hershberg, and Ficser (*7*) made a very comprehensive study of the polarographic analyses of ketosteroids. A great portion of the work done by these authors concerned the determination of the Girard T derivatives of the saturated and unsaturated ketosteroids, although they examined several of the free Δ^4-3-keto compounds in alkaline 2-propanol solutions and their findings substantiated those of Eisenbrand and Picher.

Since that time a number of articles have been published concerning the polarographic assay of Δ^4-3-ketosteroids. Hershberg, Wolfe, and Fieser (*2*) developed an assay method for 3-hydroxy-Δ^5-steroids based on the polarographic determination of the corresponding 3-keto-Δ^4-compounds after oxidation with aluminum *tert*-butoxide. Morris and Williams (*4*) and Morris (*3*) combined chromatography and polarography in developing a procedure for the determination of blood corticoids. Zuman, Tenygl, and Brezina (*8*) proposed a system for determining total steroid content of a mixture containing testosterone, methyltestosterone, progesterone, cholestenone, and deoxycorticosterone. They also suggested conditions under which deoxycorticosterone could be determined in the presence of testosterone and progesterone in the presence of methyltestosterone.

The present work describes a polarographic method capable of assaying mixtures of a Δ^4-3-ketosteroid (cortisone) and a $\Delta^{1,4}$-3-ketosteroid (prednisone). This assay is very useful for following the microbiological transformation of cortisone to prednisone (*5*, *6*).



CORTISONE



PREDNISONE

EXPERIMENTAL

Materials. The steroid compounds were made and purified in the Schering Laboratories. All chemicals used in the polarographic solutions were of reagent quality.

Apparatus. All polarographic measurements were made with the Sargent Model XXI recording polarograph. The polarographic cell was a small H-cell (3-ml. sample volume) with an anode compartment consisting of a normal calomel electrode. The capillary constant, $m^{2/3}\,t^{1/6}$, was 1.80 mg.$^{2/3}$ sec.$^{-1/2}$ at an open circuit with the electrode immersed in a 0.1*N* potassium chloride solution.

Buffer Solution. The stock buffer solution was prepared by dissolving 1.0 mole of anhydrous sodium acetate in 1.0*M* acetic acid in a 1.0-liter volumetric flask and then diluting to volume with more 1.0*M* acetic acid.

Basis for Procedure. Both prednisone and cortisone give well-formed, reproducible polarographic waves (Figure 1) in a 50% methanol solution buffered at pH 5.5 with sodium acetate–acetic acid buffer. The position of the waves ($E_{1/2}$ = −1.20 and −1.36 volts for prednisone and cortisone, respectively) is independent of concentration and the wave heights are linearly dependent on concentration over fairly wide concentration ranges. Because of the wave separation it seemed likely that mixtures of the compounds might be determined. However, on examining the individual polarograms it was evident that the wave of one compound in a mixture would encroach on the wave of the other. To correct for this overlapping, equations were set up that would account for the current of a mixture at each of two voltage points corresponding to voltages near the plateau of each compound.

$$k_1x + k'_1y = i_1 \tag{1}$$

$$k_2x + k'_2y = i_2 \tag{2}$$

where k and k' are the current constants (i_d/C) for prednisone and cortisone, respectively. The subscripts 1 and 2 refer to the two voltage points, −1.31 volts (prednisone plateau), and −1.47 volts (cortisone plateau). The following values for x and y, the concentration of prednisone and cortisone, respectively, are obtained:

$$x = \frac{i_1k'_2 - i_2k'_1}{k_1k'_2 - k_2k'_1} \tag{3}$$

$$y = \frac{k_1i_2 - k_2i_1}{k_1k'_2 - k_2k'_1} \tag{4}$$

The use of these equations should yield fairly accurate results in spite of small amounts of wave encroachment. The results of further experimentation showed this to be so.

General Procedure. All polarographic sample solutions were made by weighing the desired amount of steroid into a 10-ml. volumetric flask on a microbalance. This material was dissolved in 5 ml. of methanol, 1 ml. of buffer was added, and the solution was diluted to volume with water. Two portions of the sample solution were used to rinse the cell and a third portion was deaerated with nitrogen for 10 minutes before polarographing. All current measurements were made by the point increment method with observations at −0.90 (zero point), −1.31 (prednisone plateau), and −1.47 (cortisone plateau) volts *vs.* N.C.E. Sample currents were corrected for a blank solution measured at the same voltages. The temperature was maintained at 25° ± 0.2° C.

Determination of Prednisone-Cortisone Mixtures. Several samples of prednisone and cortisone (0.5 mg. per ml.) were polarographed in the above manner and current constants obtained for each compound at each of the two plateau points. A number of synthetic mixtures were then made by weighing a small amount of prednisone and cortisone into the same flask in such quantities that the total steroid content of the final solution was approximately 1 mg. per ml. These mixtures were examined at the three designated voltage points and the prednisone and cortisone concentrations determined by substitution of the currents obtained into the equations derived earlier. The experimental results are compiled in Table I.

Determination of Mixtures High in One Component. It was observed experimentally that the current constants for both prednisone and cortisone at low concentrations were substantially higher than those used in the above work. Any change in the actual current-concentration relationship from that used to set up the equations would, of course, lead to errors in the determination. Fortunately, the deviations from linearity for each compound occur at low solute concentrations, and current error introduced by the higher current per unit concentration value of the steroids in this region is small relative to the total current involved. For this reason the maximum absolute error introduced in a determination is from 3 to 4% of the major component. However, with material rich in one of the components, the method can be made to yield more accurate results.

For this work solutions of 1.0 and 0.1 mg. per ml. of both cortisone and prednisone were run and current constants determined for the compounds at the appropriate voltages. One series of synthetic mixtures was made up containing 90 to 100% prednisone and another series was made up containing 90 to 100% cortisone. These mixtures were then assayed using the special current constants obtained above (Table II). For the mixtures rich in prednisone the constants obtained for the concentrated prednisone solution and the dilute cortisone solution were used. For the mixtures rich in cortisone the opposite constants were used.

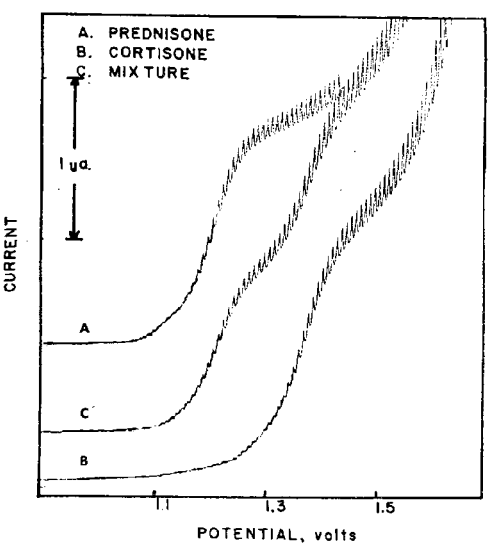


Figure 1. Polarograms of prednisone, cortisone, and a mixture of the two compounds in 50% methanol at pH 5.5

Precision. A synthetic mixture of prednisone and cortisone was dissolved in methanol and precipitated, then dried, and ground to a powder. Ten separate samples of this mixture were weighed and the components determined (Table III).

ANALYSIS OF RESULTS

The experimental results indicate that both components of a prednisone-cortisone mixture can be determined polarographically. If the standard current constants are determined from solutions whose steroid concentration is one half that of the sample solutions assayed, the prednisone-cortisone content can be determined with a maximum error of 4% absolute in the major component and 2% in the minor component. More accurate results can be obtained for the mixtures rich in one component by the application of special current constants. A prednisone-cortisone mixture can be assayed with a precision within ±0.8% (1 sigma).

Table I. Analysis of Prednisone-Cortisone Mixtures

Prednisone Concn., Mg./Ml.	Cortisone Concn., Mg./Ml.	% Prednisone Found	% Prednisone Calcd.	% Cortisone Found	% Cortisone Calcd.
1.005	0.000	96.8	100.0	−0.7	0.0
0.937	0.105	86.8	89.9	11.0	10.1
0.815	0.206	78.8	79.8	20.0	20.2
0.740	0.307	68.7	70.7	30.2	29.3
0.613	0.408	58.2	60.0	41.1	40.0
0.503	0.503	51.6	50.0	49.3	50.0
0.306	0.761	29.0	28.7	70.6	71.3
0.213	0.805	21.3	20.9	78.7	79.1
0.105	0.919	10.9	10.3	86.0	89.7
0.000	1.009	−0.6	0.0	96.0	100.0

Current constants obtained from samples containing 0.5 mg. per ml. of prednisone and cortisone.

Table II. Analysis of Prednisone-Cortisone Mixtures

Prednisone Concn., Mg./Ml.	Cortisone Concn., Mg./Ml.	Prednisone, % Found	Prednisone, % Calcd.	Cortisone, % Found	Cortisone, % Calcd.
1.0 Mg./Ml. Prednisone, 0.1 Mg./Ml. Cortisone					
1.005	0.000	100.8	100.0	−0.8	0.0
1.217	0.040	95.9	96.8	2.1	3.2
1.101	0.060	94.7	94.8	4.0	5.2
1.005	0.100	91.0	91.0	8.1	9.0
0.908	0.100	89.9	90.1	8.8	9.9
0.1 Mg./Ml. Prednisone, 1.0 Mg./Ml. Cortisone					
0.000	1.009	−1.0	0.0	100.0	100.0
0.020	1.009	2.6	1.9	97.2	98.1
0.037	1.014	3.5	3.8	96.5	96.2
0.080	1.124	5.9	6.6	92.4	93.4
0.100	1.018	8.5	8.9	90.7	91.1

Table III. Precision of Method

Sample Concn., Mg./Ml.	Prednisone, %	Cortisone, %
0.520	86.9	11.7
0.510	87.5	12.7
0.502	87.1	13.5
0.536	87.3	12.7
0.504	87.7	13.1
0.505	87.7	12.3
0.517	88.4	12.4
0.501	89.6	12.0
0.537	88.1	11.9
	Mean 87.8	12.4
	Std. dev. ±0.8	±0.6

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RECEIVED for review March 22, 1956. Accepted July 20, 1956. Seventh Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 28, 1956.

Spectrophotometric Determination of Rhenium

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A rapid, accurate, and sensitive spectrophotometric method for the determination of rhenium is based on the formation of hexachlororhenate(IV) ion by the chromium(II) chloride reduction of perrhenate in strong hydrochloric acid solution. The absorption maximum of hexachlororhenate at 281.5 mμ is used, and the small absorbance of the chromium at this wave length is eliminated by the use of a blank. The stoichiometry of the chromium(II) chloride–perrhenate reaction is discussed.

A SPECTROPHOTOMETRIC method for determination of rhenium, based on the formation of hexachlororhenate(IV) ion, was proposed by Meyer and Rulfs (*11*). The hexachlororhenate was produced by boiling perrhenate with a large excess of hydrazine for an hour in about 10*N* hydrochloric acid. Absorbance readings were taken at the absorption maxima for hydrazine and hexachlororhenate, and a two-component analysis was applied.

An investigation in this laboratory showed that hexachlororhenate could be produced quantitatively by the chromium(II) chloride reduction of perrhenate. A spectrophotometric method involving this reaction has two inherent advantages over the Meyer and Rulfs method. Hexachlororhenate can be produced quantitatively by chromium(II) chloride reduction in a couple of minutes, thus decreasing the time required for analysis by several-fold, and the two-component analysis can be eliminated. The contribution of chromium to the total absorbance at the hexachlororhenate maximum is small and can be simply and precisely cancelled with a blank.

This paper describes chromium(II) chloride reduction of perrhenate to hexachlororhenate, and the application of this reaction to the spectrophotometric determination of small amounts of rhenium.

EXPERIMENTAL

Reagents. The perrhenate stock solution was prepared from potassium perrhenate (University of Tennessee). The potassium perrhenate assayed 99.5% by determination with nitron (*2*).

Potassium hexachlororhenate(IV) was prepared by reduction of potassium perrhenate with hydroiodic acid in concentrated hydrochloric acid solution (*5*). The hexachlororhenate(IV) ion in the preparation assayed 99.1% by determination as cesium hexachlororhenate (*1, 12*).

Standard chromium(II) chloride solutions were prepared directly in the storage flask by the procedure described by Lingane and Pecsok (*8*). The concentration of the standard chromium(II) chloride solution was checked by titration against standard potassium dichromate in the presence of excess ferrous ions (*8*).

Apparatus. Light absorption measurements were made in 0.1-cm. and 1-cm. silica cells on the Cary Model 14M recording spectrophotometer. This instrument was convenient to use in the spectrophotometric study, because accurate measurements could be made over the entire absorbance range. The absorbance measurements at 281.5 mμ were made with a slit width of 0.07 mm.

Procedure. About 6 to 7 ml. of the solution to be analyzed, containing between 0.07 and 1.4 mg. of rhenium as perrhenate, were placed in a 50-ml. volumetric flask, and 25 ml. of concentrated hydrochloric acid (12.4*N*) were added. Nitrogen was passed through the flask while 1.00 ml. of about 0.045*M* chromium(II) chloride was added. The volumetric flask was stoppered and after a minute or two had been allowed for the reduction to be completed, air was bubbled through the solution for 2 to 3 minutes. After dilution to 50 ml., the absorbance of the solution was read at 281.5 mμ in a 1-cm. silica cell against a blank prepared in the same manner except for the exclusion of the perrhenate. The absorbance reading was converted to concentration of rhenium by using the Beer's law relationship and a value for the molar absorbancy coefficient determined previously on the spectrophotometer being used.

RESULTS AND DISCUSSION

Analytical Data. Varying known amounts of a standard potassium perrhenate stock solution were added to a series of 50-ml. volumetric flasks by means of a calibrated micrometer syringe buret. The perrhenate solutions were treated as described in the procedure. The absorbance value for each solution is presented in Table I.

Table I. Analytical Data

Rhenium, Mg.	Absorbance Reading at 281.5 mμ	Molar Absorbancy Index × 10^{-1}	Average Deviation, %
0.0721	0.100	1291	+1.3
	0.098	1266	−0.7
0.1444	0.196	1264	−0.9
	0.198	1278	+0.2
0.2165	0.296	1274	−0.1
	0.300	1291	+1.3
0.3247	0.444	1274	−0.1
	0.447	1282	+0.5
0.4330	0.589	1267	−0.6
	0.594	1278	+0.2
0.5412	0.742	1277	+0.2
	0.739	1272	−0.2
0.6495	0.887	1272	−0.2
	0.892	1279	+0.3
0.7577	1.034	1271	−0.3
	1.038	1276	+0.1
0.8660	1.184	1274	−0.1
	1.180	1269	−0.5
0.9742	1.333	1275	0.0
	1.334	1275	0.0
1.0824	1.476	1270	−0.4
	1.481	1275	0.0
1.1906	1.626	1272	−0.2
	1.622	1269	−0.5
1.2989	1.776	1274	−0.1
	1.777	1275	0.0
1.4071	1.929	1277	+0.2
	1.932	1279	+0.3
		Av. 1275	0.34

The solutions analyzed covered the entire concentration and absorbance range applicable to this procedure. The average deviation of the molar absorbancy index values from the average index over this range was 0.34%. This indicated that Beer's law was followed closely over this concentration range.

In order to check the validity of the Beer's law relationship at higher hexachlororhenate concentrations, the absorbances of higher rhenium concentrations were measured using 0.1-cm. instead of 1-cm. cells. Beer's law was found to be rigorously valid for these concentrations also.

As a further check on the reproducibility of the method, eight replicate determinations were made at each of two rhenium levels, 0.4330 and 1.0825 mg., according to the procedure described above. The average deviation of the individual absorbances from the average absorbance for the two rhenium amounts was 0.20 and 0.18%, respectively. For the smaller rhenium amount, the concentration of the chromium(II) chloride solution added

was cut to two thirds of the amount suggested in the procedure so that the error in the cancellation of the chromium contribution from the absorbance would be smaller.

For the solutions whose absorbances are tabulated in Table I, the per cent deviation of the molar absorbancy index from the average index also can be thought of as the per cent error in the determination of rhenium in the solution. Rhenium was determined in these solutions with an average deviation of 0.34%.

It is necessary to determine the molar absorbancy index on the specific instrument to be used. Values on different spectrophotometers have been found to vary by as much as 10%.

Choice of Acidity during Reduction. Figure 1 is a plot of the data obtained when chromium(II) chloride reduction of perrhenate was performed in solutions of varying hydrochloric acid concentrations.

Exactly 1 ml. of 0.045*M* chromium(II) chloride was added to each of a series of 50-ml. flasks containing 25 ml. of solution with varying hydrochloric acid concentrations and 1.082 mg. of rhenium as potassium perrhenate. After reduction, air was bubbled through the solutions for 2 to 3 minutes. Then the contents were diluted to 50 ml., including in the dilution sufficient hydrochloric acid so that the final acid concentration was 6.2*N* in each case. The absorbance of each of these solutions was measured against an appropriate blank at 281.5 mμ, the peak absorbance of hexachlororhenate. The per cent of rhenium as hexachlororhenate was calculated by interpolation from absorbances of solutions prepared from pure potassium hexachlororhenate(IV).

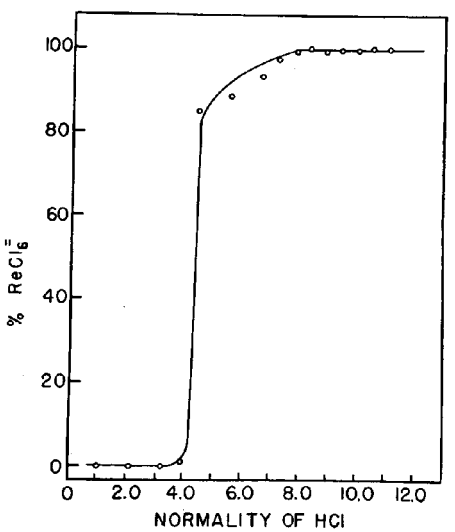

Figure 1. Yield of hexachlororhenate as a function of hydrochloric acid concentration during reduction

The plot in Figure 1 shows that practically no hexachlororhenate was present in the final solution when the hydrochloric acid concentration was below 4*N* during reduction. As the concentration of hydrochloric acid at the time of reduction increased, the concentration of hexachlororhenate increased. When the acid concentration reached about 8*N*, the yield of hexachlororhenate was 100% and higher acid concentrations caused no change.

Any hydrochloric acid concentration between 8- and 11*N* could be used for the spectrophotometric determination of rhenium. A hydrochloric acid concentration near the middle of the region was selected, so that a slight variation would not affect the yield of hexachlororhenate.

Use of a Blank. One advantage of the chromium(II) chloride reduction over the hydrazine reduction is that it does not require a two-component spectrophotometric analysis technique; the absorbance of chromium(III) ions at 281.5 mμ is small, and can be accurately canceled with an appropriate blank.

All the chromium in the blank and in the unknowns existed as chromium(III) chloride after air was bubbled through the solutions. The resulting chromium(III) chloride contributed an absorbance of only 0.06 to the total absorbance of the solution after dilution to 50 ml. By adding the same amount of chromium(II) chloride to the blank and to the unknown, the contribution of the chromium(III) ions to the total absorbance was cancelled with good accuracy. A group of ten blanks prepared in this manner all fell within an absorbance range of 0.003 unit.

The hydrochloric acid content of the solution also contributes a small amount to the total absorbance. However, if the hydrochloric acid added to the sample and to the blank differs by no more than 1 ml., its absorbance will be canceled with an error of no more than 0.001 absorbance unit.

The absorbance contribution from the zinc ions picked up from the zinc reductor was negligible.

It is important that the chromium be added to the blank in the same manner as it is added to the unknown. The chromium must not be added to the blank as the unreduced chromium(III) ions, but should be added as chromium(II) ions, just as in the solutions containing rhenium. This precaution is necessary because there are several possible species of chromium(III) ions, which have slightly different absorbancy indices. In order for the absorbance contribution of the chromium to be canceled accurately, the same chromium(III) species must be present in the blank and in the unknown.

Production of Chromium(II) Chloride. The apparatus used for storing and dispensing standard chromium(II) chloride solutions was the same as that described by Lingane and Pecsok (*8*), except that a dry ice carbon dioxide generator (*4*) was substituted for the Kipp generator. A 10-ml. buret was used in place of the 50-ml. buret, so that the 1.00 ml. suggested in the procedure could be delivered more accurately.

A simpler method, which worked almost as well, involved pouring chromium(III) chloride through a buret partly filled with amalgamated granular zinc. Then the amount of chromium(II) chloride delivered was read directly from the buret. The volume of the chromium(II) chloride delivered could not be reproduced quite as accurately as by the other procedure, because the height of the liquid column drifted slightly, owing to hydrogen evolution. However, if the zinc was well amalgamated and if the acidity of the chromium(III) chloride solution was no greater than 0.01*N*, the results were satisfactory.

The amount of chromium(II) chloride suggested in the procedure is more than is necessary to reduce easily the maximum amount of rhenium that can be determined by this procedure. The oxygen need not be removed from the original solution. It is not important that the concentration of the chromium(II) chloride solution be accurately known; but the same amount must be added to both the blank and the unknown.

Reaction Path. In order to obtain a quantitative yield of hexachlororhenate, it was necessary to expose the reduced rhenium species to air. When a large excess of chromium(II) ions was added to a strong hydrochloric acid solution of perrhenate under an atmosphere of nitrogen, absolutely no peak appeared at the absorption maximum for hexachlororhenate. However, after air was bubbled through the solution for several minutes, the absorbance at 281.5 mμ showed that quantitative formation of hexachlororhenate had occurred. This strongly indicated that the rhenium had been reduced below the quadrivalent state, and then was air-oxidized to hexachlororhenate(IV) ion.

In order to ascertain how far the perrhenate had been reduced, 0.1500 mmole of perrhenate was titrated potentiometrically with 0.1000*N* chromium(II) chloride. The titration was conducted in 9.8*N* hydrochloric acid solution, so that it would correspond to the perrhenate reduction employed in the procedure.

The titration curve gave a large break in potential after only 3.0 equivalents of chromium(II) had been added (Figure 2,*A*). This, at first, seemed to indicate that the perrhenate had been reduced only to the quadrivalent state.

In order to obtain information concerning the course of the reaction, small samples were withdrawn from the titration flask at regular intervals during a titration and replaced under the protection of nitrogen. The absorbances of these samples were measured at the absorbance peak for hexachlororhenate. From the absorbance readings, the per cent of the rhenium present as hexachlororhenate was calculated.

The amount of hexachlororhenate formed was quantitative at the end point of the titration, but fell off rapidly as excess chromium(II) chloride was added (Figure 2,*B*). Evidently, the excess chromium(II) chloride reduced the hexachlororhenate(IV) ion to a lower valence state. This view was strengthened by the fact that every sample withdrawn after the end point when exposed to air gave an absorbance reading corresponding to quantitative formation of hexachlororhenate.

The slope of the line after the end point in Figure 2,*B*, is sufficiently great so that the reduction of hexachlororhenate(IV) ion could not involve a change of 2 or more electrons. On this basis, it seems apparent that the chromium(II) chloride reduced some of the hexachlororhenate(IV) ion to rhenium(III) after the end point of the titration had been passed.

That no second break occurs in the titration curve is not proof that further reduction does not take place. Even if potential differences favored a second break in the titration curve, this stage would not appear, because at potentials more negative than about −0.2 volt, the platinum indicator electrode approximates the potential of the $H^+ - H_2$ couple. This is a result of the reaction

$$Cr(II) + H^+ = {}^1/_2H_2 + Cr(III)$$

which takes place at the surface of the indicator electrode. This would obscure the break for the second stage of the rhenium reduction.

The reported evidence concerning the stability of acid solutions of trivalent rhenium is conflicting (*3*, *6*, *7*, *9*). The findings of Manchot and Dusing (*9*) seem consistent with the results reported in this paper. They reported the rhenium(III) in strong hydrochloric acid solution was oxidized by air to hexachlororhenate(IV) ion. Their rhenium(III) was produced by cathodic reduction of potassium hexachlororhenate(IV) in 2- to 4*N* sulfuric acid with mercury and platinum electrodes.

The titration curve of Figure 2,*A*, shows that the break using conditions of the work reported here came at 3 equivalents, whereas Tribalet (*13*) under apparently similar conditions obtained a break after 4 equivalents had been added.

Oxidation of Reduced Rhenium Species. One to 2 minutes of bubbling compressed air through the solution at a slow rate was sufficient to oxidize all the reduced rhenium to hexachlororhenate(IV) ion. Extended aeration times led to no damaging effects, because of the extreme stability of hexachlororhenate toward air oxidation (*10*). Complete oxidation was also obtained after the reduced solution was left standing in the unstoppered flask for about a half hour.

It was essential that the air oxidation be conducted while the hydrochloric acid concentration was still high, before dilution to final volume. Where the hydrochloric acid concentration was less than 8*N* during aeration, the yield of hexachlororhenate was less than 100%. Aeration of a reduced rhenium solution which was 6.2*N* in hydrochloric acid produced an 82% yield of hexachlororhenate. When the hydrochloric acid concentration was 3.1*N*, the hexachlororhenate yield dropped to 24%.

When the hydrochloric acid concentration of these aerated solutions was increased to 9*N* and the aeration was repeated, the yield of hexachlororhenate did not increase. This indicated that, although the hexachlororhenate yield was not quantitative at the lower hydrochloric acid concentrations, the aeration had oxidized the remainder of the reduced rhenium to some form other than hexachlororhenate.

Interferences. The most important interfering element in the analysis of minerals and residues for rhenium is molybdenum. The authors have verified the use of the chloroform-cupferron extraction, applied by Meyer and Rulfs (*11*) for the elimination of molybdenum. This procedure can be applied directly, without modification, to the rhenium determination described in this paper.

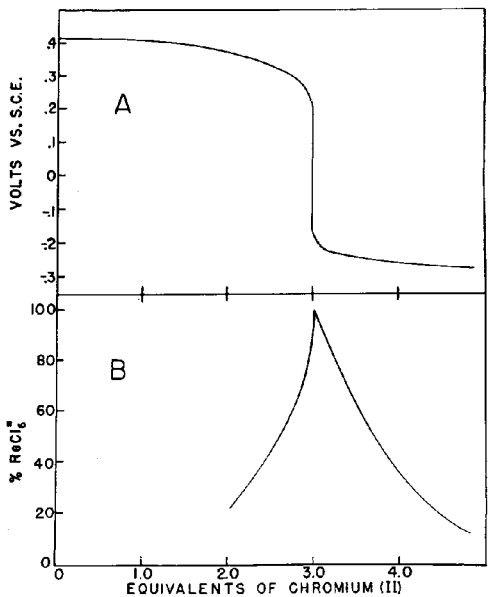

Figure 2. Effect of exposure to air

A. Titration of potassium perrhenate with chromium(II) chloride in 9.8*N* hydrochloric acid
B. % hexachlororhenate formed as function of chromium(II) chloride added

Other interferences to the rhenium determination of Meyer and Rulfs (*11*) are almost identical to those for this method. The methods described by Meyer and Rulfs for the elimination of these interferences are also applicable here.

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RECEIVED for review May 31, 1956. Accepted August 3, 1956. Work supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

p-Phenylenediamine Derivatives as Reagents for Ultraviolet Absorptiometric Determination of Nitrite Ion

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The ultraviolet spectra of the diazonium salts formed from *p*-phenylenediamine and many of its derivatives can be used for the absorptiometric determination of the nitrite ion. These salts absorb strongly in the 320- to 400-mμ region, where the reagents themselves do not absorb significantly. The sensitivity of these reagents toward nitrite ion is comparable to that of existing colorimetric methods, the molar absorbancy indices of the diazonium salts being in the 33,000 to 40,000 range. Chloro-*p*-phenylenediamine was singled out for thorough investigation of reproducibility, reagent stability, and interference effects.

THE reaction of aromatic amines with nitrous acid, sometimes referred to as the diazo reaction, has been used extensively in the colorimetric determination of nitrite ion. In these methods, the diazotization of the amine is followed by a coupling step with a suitable reagent to give a colored azo dye, the intensity of which is related to the original nitrite ion concentration. The most widely used colorimetric method for nitrite ion is that employing sulfanilic acid as the diazotee, and 1-naphthylamine as the coupling agent. Rider and Mellon (*5*) have made a critical study of this method. These reagents were proposed by Griess (*2*) in 1870, and remain today the basis of the standard method for nitrite ion sanctioned by the American Public Health Association and the American Water Works Association (*1*).

The spectrophotometric determination of nitrite ion utilizing the diazo reaction alone, without subsequent coupling of the diazonium salt, has received little attention. The primary reason for this is probably the fact that the absorption maxima of a majority of the diazonium salts lie in the ultraviolet region, necessitating special instrumentation (quartz optics, etc.) if these maxima are to be used in absorptiometric methods. The only reported method for nitrite ion utilizing the ultraviolet absorption of diazonium compounds is that of Pappenhagen and Mellon (*4*). This method is based upon the changes caused by the nitrite ion (via nitrous acid) in the absorption spectrum of sulfanilic acid at 270 mμ. Measurements were made *vs.* a reagent blank, because sulfanilic acid itself absorbs significantly at this wave length. The major disadvantage of the method is its low sensitivity (a_M = 15,300) compared to accepted colorimetric methods.

The ultraviolet method for nitrite ion offers the distinct advantage of eliminating the coupling step, with its attendant problems in pH adjustment, timing, and limited solubility of the azo dye. In an attempt to find a better reagent than sulfanilic acid for the ultraviolet determination of nitrite ion, particularly in regard to sensitivity, more than 100 aromatic amines were surveyed as to the effect of nitrous acid on their ultraviolet absorption spectra. This survey showed *p*-phenylenediamine derivatives to be exceedingly sensitive reagents toward nitrite ion (a_M = 33,000 to 40,000).

The work reported herein is a summary of a detailed investigation of *p*-phenylenediamine derivatives which followed the original survey of aromatic amines. The chloro-*p*-phenylenediamine system was singled out for investigation of reproducibility, reagent stability, and interference effects, and is discussed in some detail. To reduce the amount of repetition, the designation "para" is usually omitted and is to be assumed in all subsequent discussion of the *p*-phenylenediamines.

APPARATUS AND REAGENTS

Apparatus. Absorption spectral data were obtained on three different instruments. A Cary Model 10-11M recording spectrophotometer was used in all the preliminary work where the entire ultraviolet spectrum was wanted. All calibration curves, interference effects, and stability and precision studies were obtained using the Beckman Model B and Model DU spectrophotometers. The sensitivity control on the Beckman Model B was kept at 2 for this work. Matched 1-cm. quartz absorption cells were used with all the instruments.

Glass-stoppered volumetric flasks of 50-ml. capacity were used in the preparation of all of the solutions run on the instrument. All pH measurements were made on a Beckman Model H-2 glass electrode pH meter.

Reagents. The sodium nitrite, potassium permanganate, sodium thiosulfate, and potassium iodide used in the preparation and standardization of the stock nitrite solution were Baker's analyzed reagents. All the *p*-phenylenediamine derivatives investigated were the best grade obtainable of Eastman organic chemicals. The amine hydrochlorides were used whenever available because of the greater stability of the acid salts compared to free amines. The Eastman catalog number of the various reagents is included in the data of Table I.

Stock Solutions. Stock solutions of the various amines investigated were prepared in 0.005- or 0.01*M* concentration by dissolving an accurately weighed portion of the reagent in distilled water acidified with 1 to 4 hydrochloric acid, followed by dilution to the desired volume. The final acid concentration of the stock solutions used in the studies of the effect of pH and reagent con-

Table I. Sensitivity of *p*-Phenylenediamine Derivatives toward Nitrite Ion

Compound	Eastman Catalog No.	λ of Max. Change, Mμ	$a'M$ at $\lambda_{Max.}$	Time after Mixing, Min.	Reagent Absorption, A_s = 0.01 at Conen., Mμ	Conen., *M*	pH
H_2N-C_6H_4-NH_2	394	353	39,400	10–15	280	0.0003	1.4
H_2N-C_6H_4-$NHCH_3$	1339	365	22,300	10–20	315	0.001	1.1
H_2N-C_6H_4-$N(CH_3)_2$	192	376	33,500	13–20	350	0.0004	1.2
H_2N-C_6H_4-$N(Et)_2$	1374	378	37,700	3–23	315	0.001	1.1
H_2N-C_6H_4-$NHCOCH_3$[a]	13	335	23,500	20–120	290	0.0001	0.3
H_2N-C_6H_4-$N(CH_3)COCH_3$	T1773	338	12,200	7–15	305	0.0008	1.1
H_2N-$C_6H_3(CH_3)$-NH_2	1206	353	36,300	10–20	300	0.001	1.1
H_2N-$C_6H_3(Cl)$-NH_2	3654	354	37,900	5–100	340	0.001	1.2
H_2N-$C_6H_3(CH_3)$-$N(Et)_2$	3478	378	39,400	3– 10	300	0.0004	1.3

[a] Data given apply to system at pH 0.3.

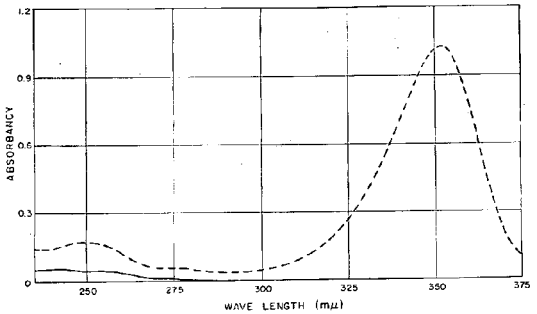

Figure 1. Effect of nitrous acid on absorption spectrum of *p*-phenylenediamine at pH 1.4

—— 0.0002*M* solution of reagent
– – – 0.0002*M* solution of reagent 10 to 15 minutes after addition of 0.059 mg. of nitrite ion per 50 ml.

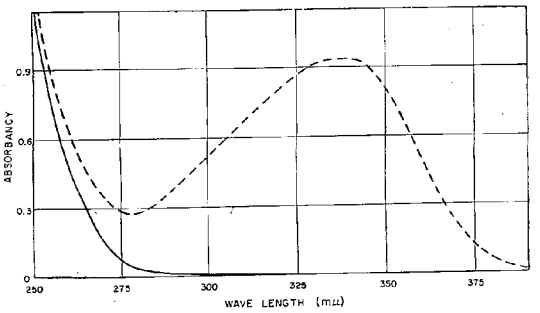

Figure 2. Effect of nitrous acid on absorption spectrum of *p*-amino-*N*-methylacetanilide at pH 1.1

—— 0.0008*M* solution of reagent
– – – 0.0008*M* solution of reagent 7 to 15 minutes after addition of 0.178 mg. of nitrite ion per 50 ml.

centration was approximately 1 to 100 or 1 to 50 hydrochloric acid (5 or 10 ml. of 1 to 4 hydrochloric acid per 100-ml. volume). Stock solutions of the amines prepared for the calibration work were usually relatively acidic (1:12 to 1:4 hydrochloric acid) solutions. The acid content of each stock solution was so adjusted that the aliquot most frequently taken would contain sufficient acid to give the desired pH (1.0 to 1.5). This eliminated the need for a separate addition of acid in preparing the test solutions to be run on the instruments. Stability studies on the reagents themselves were carried out on these acidified stock solutions, as they were generally more stable than those of the same concentration of reagent prepared in more dilute acid.

The standard nitrite solutions used throughout this work were approximately 0.00025*M* (0.0115 mg. of nitrite-ion per ml.), and were prepared by successive dilution of 0.05- and 0.01*M* solutions of sodium nitrite. The 0.05*M* nitrite solution was standardized titrimetrically, by adding an excess of potassium permanganate to oxidize the nitrite, followed by a thiosulfate titration of the iodine liberated by the action of the excess permanganate on potassium iodide. Details of this standardization procedure are given by Kolthoff and Sandell (*3*). The exact concentration of the 0.00025*M* nitrite solution was calculated from the standard value of the 0.05*M* solution, using the necessary dilution factors.

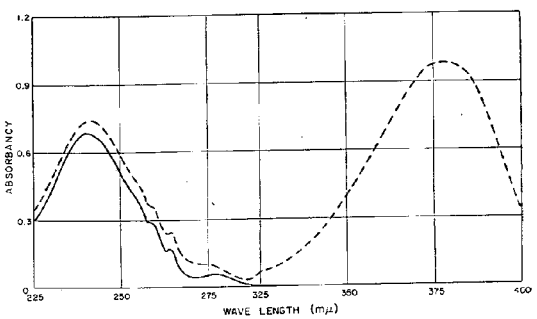

Figure 3. Effect of nitrous acid on absorption spectrum of *N,N*-diethyl-*p*-phenylenediamine at pH 1.1

—— 0.001*M* solution of reagent
– – – 0.001*M* solution of reagent 3 to 23 minutes after addition of 0.059 mg. of nitrite ion per 50 ml.

SURVEY OF PHENYLENEDIAMINES

Effect of Nitrous Acid on Absorption Spectra. Figures 1 to 3 illustrate the type of absorption changes which takes place upon the addition of nitrite ion to phenylenediamine derivatives in acid solution. All of the compounds investigated give very similar changes. The principal absorption maxima of all the diazonium salts occur in the 330- to 380-mμ region, whereas the reagents themselves show no significant absorption.

Table I summarizes the ultraviolet spectral data for the various reagents. The data apply to systems having pH's of 1.0 to 1.4, unless otherwise stated. The sensitivities of the various reagents toward nitrite ion are expressed as molar absorbancy indices (a_M values). These values were calculated according to the equation, $a_M = \Delta A_s(2300)/c$, where ΔA_s is the absorbancy change of the system at a specific wave length upon the addition of c mg. of nitrite ion per 50 ml. and 2300 is a factor converting the nitrite concentration to moles per liter.

The ultraviolet cutoffs of the reagents themselves are also listed in Table I. The wave length at which the reagent starts to absorb significantly ($A_s = 0.01$) is given, together with the reagent concentration and pH of the solution to which the cutoff applies.

Stability of the Systems. The diazonium salts formed from the *p*-phenylenediamines are light-sensitive. If no precautions are taken to shield the solutions from light after mixing, the diazonium salts decompose a few minutes after they are formed, as is evidenced by the rapid decrease in the intensity of the absorption maxima in the 330- to 380-mμ region. The absorbancy obtained depends upon how long the solutions were exposed to the light before they were placed in the spectrophotometer.

If the solution is transferred to the absorption cell and placed in the cell compartment immediately after mixing, an extremely stable system results. Under these circumstances, the absorbancy quickly rises to a maximum and remains constant if the solution is not exposed to the light. All the work reported herein pertains to solutions placed in the instrument immediately after mixing. The time intervals specified in Table I pertain to the time after mixing during which the systems were found to give a constant absorbancy reading, if kept in the darkness of the spectrophotometer cell compartment. No formal stability studies of the various systems were undertaken for periods longer than those specified. However, it was apparent during the course of the investigation that the majority of the systems are stable for hours if light is excluded.

Effect of pH and Reagent Concentration. The effect of pH and reagent concentration of the system was determined in some instances. Varying the pH in the range 1.0 to 2.4 usually changed the spectrum of the reagent considerably, but had little or no effect on the intensity of the absorption maximum of the diazonium salt in the 330- to 380-mμ region. In general, it was observed that both the formation and light-catalyzed decomposition of the diazonium salt proceed at slower rates as the pH is raised. Perchloric and sulfuric acid were substituted for hy-

drochloric acid in some of the tests involving phenylenediamine and 2,5-toluenediamine. The use of these acids resulted in systems which gave less intense absorption maxima, and which reached these maxima more slowly than the corresponding systems in hydrochloric acid.

The concentration of several of the reagents was varied from 0.0002- to 0.001- or 0.002*M*, with negligible effects upon the intensity of the absorption maxima of the diazonium salts.

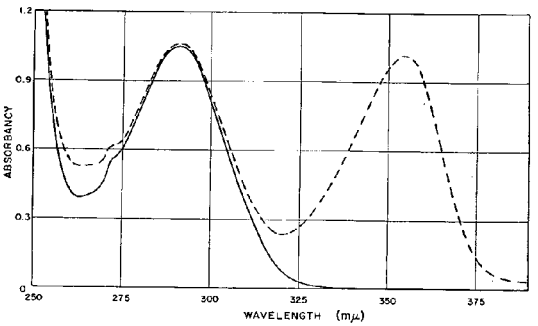

Figure 4. Effect of nitrous acid on absorption spectrum of chloro-*p*-phenylenediamine at pH 1.2

—— 0.001*M* solution of reagent
– – – 0.001*M* solution of reagent 3 to 11 minutes after addition of 0.058 mg. of nitrite ion per 50 ml.

Stability of the Reagents. Aromatic amines in general are sensitive to air and light, and should be protected therefrom as much as possible. The amine hydrochlorides were chosen for this work, when available, so as to minimize the decomposition which often occurs in amino compounds upon prolonged standing.

Stability studies were carried out on hydrochloric acid solutions of several of the reagents. A 0.0075*M* solution of phenylenediamine in 1 to 4 acid and a 0.05*M* solution in 1 to 50 acid were found to be stable for 48 hours. The less acidic solution showed a more rapid rate of decomposition after this period.

A 0.01*M* stock solution of *N,N*-diethylphenylenediamine in 1 to 12 acid was found to be stable for 7 days after its preparation. It gave reproducible absorbancy readings upon the addition of nitrite ion during this period, despite the development of a slight pink hue in the solution after 3 or 4 days.

A 0.001*M* solution of aminoacetanilide in 1 to 50 acid gives reproducible absorbancy readings for 5 days after its preparation. Stock solutions 0.01*M* in 2,5-toluenediamine, in both 1 to 100 and 1 to 4 hydrochloric acid, showed signs of decomposition 2 to 3 days after their preparation.

Mole Ratio Studies. The similarity in absorption characteristics among the various systems investigated is apparent from Table I. Compounds having *N*-substituted amino groups give the same type of absorption maximum, in the same region and of comparable intensity to those having two free amino groups. This would indicate that only one of the free amino groups is involved in the reaction with nitrite ion, and that diazotization and not tetrazotization is the predominant reaction.

To confirm this belief, mole ratio studies were carried out on the phenylenediamine, chlorophenylenediamine, and 2,5-toluenediamine systems. The data in Figure 5 pertaining to chlorophenylenediamine are indicative of the type of plot obtained in these studies, and the range of concentrations employed. Values of 0.92 and 0.87 mole of nitrite per mole of reagent were obtained for the phenylenediamine system. 2,5-Toluenediamine gave 0.90 and 0.82 mole of nitrite per mole of reagent. Results of the mole ratio studies using chlorophenylenediamine are discussed in the following section.

Calibration Curves. The Model DU spectrophotometer was calibrated for the determination of nitrite ion using several of the reagents. Details of the calibration procedure will be found in the following section. The phenylenediamine, chlorophenylenediamine, 2,5-toluenediamine, and *N,N*-diethylphenylenediamine systems all followed Beer's law at the wave lengths given in Table I up to the calibration limit of 0.06 mg. of nitrite ion per 50 ml. Slit widths of 0.08 to 0.11 mm. were used in this work on the Model DU.

The Beckman Model B spectrophotometer was also calibrated using a few of the reagents. The aminoacetanilide system follows Beer's law over the range 0.00 to 0.08 mg. of nitrite ion per 50 ml. when this instrument is used. The *N,N*-diethylphenylenediamine system shows a slight deviation from the usual straight-line plot above 0.04 mg. of nitrite ion per 50 ml., in contrast to its behavior using the Model DU.

CHLOROPHENYLENEDIAMINE SYSTEM

Variables Influencing Absorption Spectrum. The effect of nitrous acid on the absorption spectrum of chlorophenylenediamine is shown in Figure 4. The addition of nitrite ion causes absorption changes similar to the phenylenediamine derivatives discussed in the previous section. Varying the pH from 1.2 to 2.4 causes a decrease of 0.06 absorbancy unit in the 354-mμ maximum at the absorbancy level shown in Figure 4. A change of 0.02 absorbancy unit in this maximum is also observed when the reagent concentration is varied from 0.0002- to 0.001*M*.

The changes noted above indicate that the system is not entirely insensitive to pH and reagent concentration. However, these variables cannot be considered critical, since slight deviations from the specified conditions in any proposed method cause negligible error as far as pH and reagent concentration are concerned.

All the data presented in this section refer to the behavior of the chlorophenylenediamine system in the absence of light, as this system exhibits the same light instability as other phenylenediamine derivatives.

Stability of the Reagent. The reagent, a white free-flowing powder, was obtained as the dihydrochloride. The presence of the electronegative chlorine atom on the ring was expected to increase the stability of this compound compared to phenylenediamine itself, or its derivatives containing alkyl groups on the nitrogen or benzene ring. A 0.005*M* solution of the reagent in 1 to 100 hydrochloric acid is colorless, but develops a faint

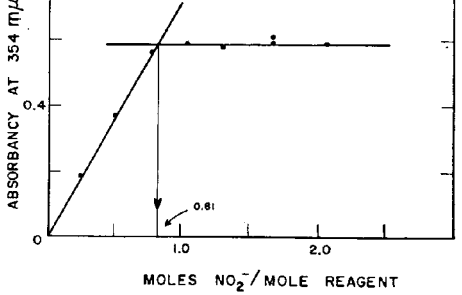

Figure 5. Mole ratio plot for chloro-*p*-phenylenediamine system at 354 mμ

Instrument. Beckman Model B, slit width approximately 0.8 mm.
Reagent concentration. $2 \times 10^{-5}M$
Nitrite concentration. 0.51×10^{-5} to $4.08 \times 10^{-5}M$
Acidity. pH 1.2

pinkish brown tint after standing for 15 days. Qualitatively, this is indicative of greater stability, since stock solutions of many of the other diamines in this series developed pink hues after 2 or 3 days. No quantitative stability studies were undertaken on this weakly acidic solution.

Table II. Calibration Curve Data and Results of Precision Studies for Chloro-*p*-phenylenediamine System

Reagent Concn. 0.001*M*
pH. 0.9 to 1.1

Nitrite Ion Concn., Mg./50 Ml.	A_s at 354 Mμ Range	A_s at 354 Mμ Average	n[a]	Standard Deviation of A_s at 354 Mμ[b]
0.0117	0.190–0.195	0.192	5	0.0021
0.0234	0.369–0.388	0.378	19	0.0054
0.0351	0.552–0.594	0.578	42	0.0093
0.0468	0.752–0.785	0.774	11	0.0105
0.0585	0.939–0.996	0.972	21	0.0127

[a] n = number of test solutions used to obtain data at each level of nitrite ion concentration.
[b] $\sigma = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$.

Stability studies were carried out on several acidified 0.01*M* stock solutions of the reagent. These solutions were prepared by dissolving 0.2155-gram portions of the reagent in 40 ml. of water and 60 ml. of 1 to 4 hydrochloric acid, giving a final acid concentration equivalent to approximately 1 to 7 hydrochloric acid. The stability of these solutions was indicated by the constancy of absorbancy readings obtained with the same quantities of nitrite ion over the period studied. The stability of three different stock solutions investigated at different times during a 3-month period varied from 5 to 8 days. No color was observed in any of the stock solutions during these studies. The reason for the variation in stability is not definitely known. The stock solutions were kept in the dark when not in use. It is possible that they were exposed to unequal amounts of light during the first few days after their preparation, and hence showed different rates of deterioration. It is recommended that 5 days be taken as the maximum period during which any given 0.01*M* reagent stock solution can be used.

Mole Ratio Studies. On the basis of previous work with other phenylenediamine derivatives, the species accounting for the absorption maximum at 354 mμ was again believed to be the diazonium salt rather than the tetrazonium compound. To support this contention, two mole ratio studies were carried out on this system. One study involved varying the nitrite ion concentration while keeping the concentration of reagent constant, and gave a value of 0.81 mole of nitrite ion per mole of reagent. The plot of the data obtained in this study is given in Figure 5. In the second study, the nitrite ion concentration was kept at a constant level while the reagent concentration was varied. A value of 1.28 moles of reagent per mole of nitrite ion was obtained. This value agrees well with the value of 1.23, calculated by taking the reciprocal of the 0.81 ratio of nitrite ion to reagent obtained in the first study.

All solutions were kept in the darkness of the cell compartment until the absorbancy reached a maximum and remained constant. This took 2 to 3 hours for some of the test solutions.

Calibration Curve. Both the Beckman Model B and Model DU spectrophotometers were calibrated for the determination of nitrite ion up to 0.06 mg. per 50 ml., using the chlorophenylenediamine system at 354 mμ. A 0.11-mm. slit width was used on the Model DU, and an approximately 0.8-mm. slit on the Model B. The system followed Beer's law when either instrument was used, and practically identical absorbancy readings were obtained on both instruments for each of the five calibration solutions. These solutions were prepared by adding 5 ml. of the 0.01*M* acidified stock solution of the reagent to various aliquots (1 to 5 ml.) of the stock nitrite solution, followed by dilution to 50 ml. The solutions were transferred to the absorption cell and placed in the spectrophotometer immediately after mixing. Absorbancy readings were taken 5 to 10 minutes after the solutions had been placed in the instrument. Molar absorbancy indices of the absorbing species at 354 mμ varied from 37,400 to 38,200 for these solutions, with an average value of 37,900. Calibration curve data are combined in Table II with the results of the precision studies.

Precision Studies. After the calibration curve had been set up, the calibration was checked repeatedly over a 3-month period using the Model B spectrophotometer. The variation in the absorbancy readings obtained for a given amount of nitrite during this period gives an idea of the reproducibility of the system, assuming negligible errors in reproducing instrument variables. Nine different 0.01*M* reagent stock solutions and four different 0.00025*M* stock nitrite solutions, prepared as described earlier, were used over the 3-month period. The same aliquots (1 to 5 ml.) of the stock nitrite solution used in the calibration work were taken for the precision studies. Table II summarizes the absorbancy readings obtained for the five different nitrite ion concentrations used.

The stock nitrite solutions were carefully prepared from the same bottle of sodium nitrite, and upon standardization were found to have the same strength. However, it must be emphasized that the chlorophenylenediamine system is more sensitive to small changes in nitrite concentration than the titrimetric method used to standardize the nitrite stock solutions. Thus, small differences in nitrite ion concentration among the four stock solutions, not detected by the method of standardization, contributed to the variation in absorbancy readings noted in Table II.

The limits of reproducibility of the Model B spectrophotometer itself for absorbancies above 0.50 unit must also be kept in mind when the precision of the chlorophenylenediamine system is evaluated.

Table III. Effects of Suspected Interfering Ions

Reagent Concn. 0.001*M*
Nitrite Concn. 0.0351 mg./50 ml.
pH. 1.0 to 1.1
Instrument. Beckman Model B, 354 mμ

Ion	Added as	Amount of Ion, Mg./50 Ml.	Relative Error, %	Max. Amount for <2% Error, Mg./50 Ml.
		Cations		
Ba^{++}	$BaCl_2.2H_2O$	25	+ 1.1	25
Be^{++}	$Be(NO_3)_2$	25	+10.5	6
Ca^{++}	$CaCl_2$	25	− 0.3	25
Cd^{++}	$2CdCl_2.5H_2O$	25	− 0.6	25
Cu^{++}	$CuSO_4.5H_2O$	25	+ 1.1	25
Hg^{++}	$HgCl_2$	25	− 0.6	25
Fe^{++}	$FeSO_4.7H_2O$	25	− 8.8	3
Fe^{+++}	$FeCl_3.6H_2O$	0.5	+12.5	0
K^+	KCl	25	− 0.6	25
Mg^{++}	$MgCl_2.2H_2O$	25	+ 1.4	25
Mn^{++}	$MnSO_4.7H_2O$	25	+ 1.7	25
NH_4^+	NH_4Cl	25	+ 0.6	25
Ni^{++}	$NiCl_2.6H_2O$	25	+ 0.9	25
UO_2^{++}	$UO_2(C_2H_3O_2)_2$	25	+ 0.6	25
Zn^{++}	$ZnCl_2$	25 —	− 0.3	25
		Anions		
BO_3^{---}	H_3BO_3	25	+ 1.1	25
Br^-	KBr	25	− 0.6	25
$C_2H_3O_2^-$	$NaC_2H_3O_2.3H_2O$	25	+ 0.3	25
$C_2O_4^{--}$	$Na_2C_2O_4$	25	+ 0.3	25
ClO_3^-	$KClO_3$	25	0.0	25
F^-	NaF	25	+ 0.9	25
I^-	KI	25	− 3.1	10
NO_3^-	KNO_3	25	+ 1.4	25
PO_4^{---}	KH_2PO_4	25	+ 0.6	25
SCN^-	KSCN	25	− 3.4	10
SO_3^{--}	Na_2SO_3	5	−16.0	0
SO_4^{--}	$(NH_4)_2SO_4$	25	+ 0.6	25
$S_2O_3^{--}$	$Na_2S_2O_3.5H_2O$	25	−98[a]	0
$S_2O_8^{--}$	$K_2S_2O_8$	25	+ 1.7	25[b]
SiO_3^{--}	$Na_2SiO_3.9H_2O$	25	+ 1.1	25
VO_3^-	$NaVO_3$	0.5	+ 5.1	0
WO_4^{--}	$Na_2WO_4.2H_2O$	1	+ 2.4	0.5

[a] Precipitate forms on standing.
[b] No interference at this level if read immediately.

Considering the inherent but unknown errors mentioned above, the data in Table II indicate that good precision can be obtained within the chosen concentration range.

Interference Effects. As part of the development of chlorophenylenediamine as an analytical reagent for nitrite ion, the effect of extraneous ions on the system was determined. This reagent was the only phenylenediamine derivative for which such interference studies were carried out. The procedure used in this work was to compare the absorbancies of the solutions containing the extraneous ions with that given by a solution of the same nitrite ion concentration containing no added ion. The absorbancy difference between the two solutions was referred to the calibration curve previously described to obtain the deviation in nitrite concentration in the presence of the diverse ion. Table III summarizes the effects of suspected interfering ions.

The usual concentration of the extraneous ion was 25 mg. per 50 ml. and that of the nitrite ion 0.0351 mg. per 50 ml. A 2% error in the nitrite ion concentration (0.0007 mg.) was taken as a tolerable limit. If the original 25 mg. of added ion caused an error greater than 2%, the amount of ion added was reduced until the error was below the 2% tolerance. These interference studies were carried out over a relatively short period of time (7 days), using the same stock nitrite solution throughout. Under these conditions, the precision of the absorbancy measurements at the 0.0351-mg. level of nitrite concentration was better than that indicated in Table II. An error in the nitrite ion concentration greater than 2% was therefore considered a significant effect of the added ion.

Recommended Procedure. Adjust the aqueous solution containing nitrite ion, obtained by suitable previous preparative treatment of the sample, to a pH between 5 and 7. The volume of the solution should be 45 ml. or less after pH adjustment. Add 5 ml. of the 0.01*M* solution of chlorophenylenediamine (in 1 to 7 hydrochloric acid), dilute to 50 ml., and mix. Transfer the sample immediately to the absorption cell, and place in the instrument. After 10 minutes, read the absorbancy at 354 mμ against a reagent or water blank, whichever was used in the calibration of the instrument. The nitrite ion content of the sample is obtained by referring the absorbancy at 354 mμ to a calibration curve, previously constructed by carrying solutions of known nitrite ion content through the same procedure.

DISCUSSION

All the reagents listed in Table I could be used for the absorptiometric determination of nitrite ion. *N*-methylphenylenediamine, aminoacetanilide, and amino-*N*-methylacetanilide were included in the table because of the similarity of their absorption characteristics to the other compounds investigated, although their sensitivities toward nitrite ion are considerably lower.

The low sensitivity of *N*-methylphenylenediamine compared to *N*,*N*-dimethylphenylenediamine and phenylenediamine itself is particularly surprising. Varying the pH, reagent concentration, and nitrite ion concentration did not affect the sensitivity significantly. It is possible that the unsymmetrical nature of the methylamino group lowers the probability that the diazonium salt will assume the resonance structure responsible for the absorption in the 330- to 380m-μ region.

The sensitivities of the reagents toward nitrite ion, calculated according to the equation given earlier, are the same as the molar absorbancy indices of the diazonium salts because of the 1 to 1 stoichiometry of the diazo reaction.

The major disadvantage of the light instability of the phenylenediamines is that samples must be prepared individually for measurement. Dilution, mixing, and transferring of the sample to the instrument must follow immediately after the addition of the reagent to the sample. The convenient and time-saving procedure of adding the reagent to a number of samples before making the final dilution and mixing cannot be used with these systems. However, the ultraviolet method eliminates the coupling step used in the colorimetric methods for nitrite ion, and hence the over-all time required per sample is approximately the same for both methods.

Although deviating considerably from the theoretical ratio of 1.00, the values obtained from the mole ratio studies indicate diazotization to be the predominant reaction. If a greater excess of reagent over nitrite ion (or nitrite ion over reagent) had been used, more complete conversion into the diazonium compound would be expected, resulting in mole ratios correspondingly closer to the theoretical. Reagent nitrite ion ratios of 40 to 1 or better are used in the proposed chlorophenylenediamine method, and give higher absorbancy readings compared to those obtained in the mole ratio studies for the same amount of nitrite ion.

Calibration curves were constructed for those systems which appeared to offer the best potential for the determination of nitrite ion. Sensitivity, reagent absorption, and reagent and system stability were the factors weighed most heavily in determining potential usefulness of the reagent. Chlorophenylenediamine was chosen for further development because of its sensitivity, ease of weighing, and the stability of the reagent, both in the solid state and in acid solution. *N*,*N*-diethylphenylenediamine was an alternative choice offering almost equal advantages.

As nitrite ion can be both oxidized and reduced, oxidizing and reducing agents would be expected to affect the system and cause interference. Most of the ions which interfere are ions that are not compatible with nitrite ion, and hence either would not be present with the nitrite ion or would destroy the nitrite ion if it were originally present. Because of their oxidizing or reducing nature, the majority of the ions found to interfere with the chlorophenylenediamine system would be expected to interfere with the determination of nitrite, regardless of the phenylenediamine derivative used.

The vanadate and tungstate ions interfere primarily because of their own strong absorption in the near-ultraviolet region. Because the phenylenediamines themselves have negligible absorption in the 330- to 380-mμ region, interferences such as these can be eliminated by determining the absorbancy of the sample at the desired wave length before and after the addition of the reagent. The absorbancy of the diazonium salt can then be corrected for the background absorption. Both samples would have to be at the same pH used in the calibration, however. The tungstate ion also appears to give some reaction with chlorophenylenediamine, as does the ferric ion. In addition to causing large errors in the absorbancy, some of the interfering ions notably decrease both the rate of formation of the 354-mμ absorption maximum and the stability of the system in general.

Besides their sensitivity and stability (if light is excluded), one of the greatest advantages of the phenylenediamine derivatives is the position of the absorption maxima of the diazonium salts. The occurrence of the maxima in the 330- to 380-mμ region permits the use of instruments having glass optics, such as the Beckman Model B. It is believed the ultraviolet absorptiometric determination of nitrite ion using phenylenediamines, particularly chlorophenylenediamine, offers many advantages over the widely used colorimetric methods.

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RECEIVED for review April 25, 1956. Accepted July 2, 1956.

Rapid Procedure for Estimation of Amino Acids by Direct Photometry on Filter Paper Chromatograms

Estimation of Seven Free Amino Acids in Orange Juice

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An improved procedure is presented for the direct photometric estimation of free amino acids on small-scale filter paper chromatograms. The estimation of total spot density is facilitated by a device for mounting the paper chromatograms in the sample chamber and in front of the photocell of the photometer. Complete amino acid assays are obtained within a 24-hour period. The assay range is 0.1 to 1.0 γ for solutions containing as little as 150 γ per ml. Values are presented for alanine, γ-aminobutyric acid, arginine, aspartic acid, glutamic acid, and proline in the filtered juices of California Valencia and Washington Navel oranges.

THE direct estimation of amino acids on filter paper chromatograms is more convenient, more rapid, and usually more accurate than indirect methods based on elution of the amino acids from the paper followed by the analysis of the eluate by established techniques. A number of variations have been suggested for the direct photometric estimation of amino acids on paper chromatograms. These include procedures employing the measurement of maximum spot density (*3, 7, 23, 29, 40, 42*); maximum spot density × spot area (*3, 4, 40*); reflectance (*18*); total spot density by scanning and curve integration (*4, 9, 10, 15, 33*); and total spot density in a single operation (*26, 30, 36*).

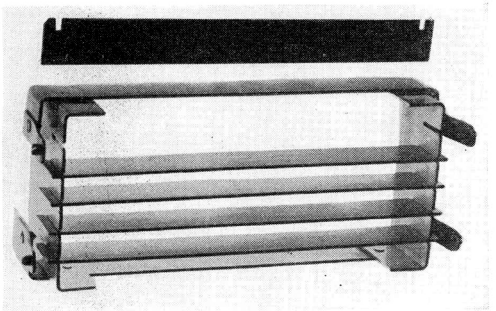

Figure 1. Stainless steel rack and mounting bars for filter paper

The limitations of the maximum density procedure have been discussed in several recent publications (*5, 29, 33*). The most reliable results are obtained with this procedure when the spots are round or elliptical. However, asymmetric spots are frequently obtained with standard solutions and particularly with extracts of natural products. Human error in ascertaining the point of maximum density may introduce further uncertainties in the analytical results. The method employing maximum density × area appears to have somewhat greater reliability. However, additional manipulations are required and errors may be introduced in circumscribing the areas occupied by lightly colored, diffuse spots. The total color density techniques appear to be capable of greater accuracy than the other direct photometric procedures. The scanning–total density procedure may be employed for the estimation of compounds which are incompletely separated on paper chromatograms (*33*). However, a greater number of manipulations are required than for the techniques which determine the color density of a whole spot in a single, rapid operation.

A variety of techniques (*13, 22, 35, 37, 38, 41*) have been described for the separation and qualitative estimation of amino acids on sheets or strips of filter paper considerably smaller than those first employed by Consden, Gordon, and Martin (*12*).

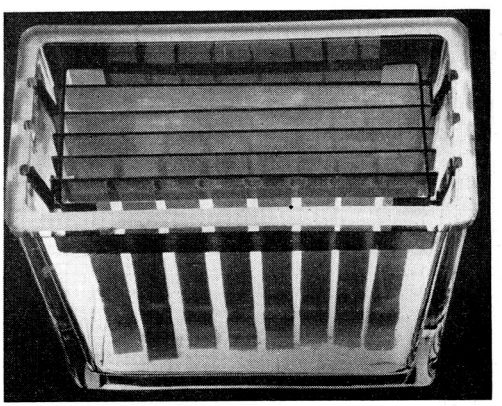

Figure 2. Arrangement of stainless steel rack and one row of filter paper strips

However, very little work has been done to adapt small-scale paper chromatography procedures to the quantitative estimation of amino acids (*6, 26, 36*). Irrigation periods varying from 1 to 5 days or more are required for the separation of amino acids on large, conventional types of paper chromatograms. When employed for quantitative purposes, the use of large chromatograms may impose the problem of maintaining a relatively large space at a constant and uniform temperature, as well as a limitation upon the number of replicate chromatograms that may be irrigated at the same time under identical conditions. The use of small-scale chromatographic procedures obviates these complications without imposing further limitations. With any given solvent and unit quantities of solutes, it is to be expected that the resolution of solute would be improved as the solvent migration distance is increased. However, when the amounts

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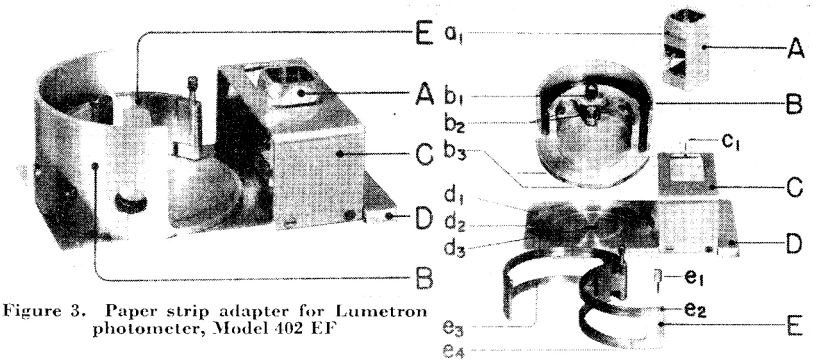

Figure 3. Paper strip adapter for Lumetron photometer, Model 402 EF

A. Periscope to center spot in slit
a_1. Support
B. Turret
b_1. Geared drive to rotate paper strip holder
b_2. Two of the five slits
b_3. Position notches for turret
C. Periscope mounting bracket
c_1. Slot along which periscope is moved out of incident light beam
D. Base plate
d_1. Recess for turret
d_2. Spring latch to engage turret notches
d_3. Central pivot slot into which stem (not shown) on turret is recessed
E. Paper strip holder
e_1. Locking pin
e_2. Locking pin insert
e_3. Recess on inner side to accommodate paper strips
e_4. Radial gear teeth

of solutes and the initial spot sizes are drastically reduced, a shorter solvent travel distance is required to effect the same resolution (*2, 11*). Because the time required for a solvent to ascend a unit distance on a given filter paper is proportional to the square of the solvent travel distance (*2, 27, 46*), it is practicable to employ slower moving, more effective solvents for small-scale chromatography than for conventional chromatographic procedures.

The small-scale, direct photometric technique suggested by Rockland and Dunn (*36*) is rapid and convenient for the analysis of a large number of samples. The original procedure, which employed trapezoidal strips of filter paper irrigated in individual test tubes (*37*), has been modified to permit the simultaneous irrigation of 40 individual rectangular paper strips in a single chamber. The photometric measurement of total spot density on paper chromatograms has been facilitated by an improved device for mounting the paper strips in front of the photocell. Compared with conventional large-scale procedures which may require 2 to 7 days, complete amino acid assays may be obtained by the present small-scale procedure within a 24-hour period. The assay range for small-scale chromatography is generally from 0.1 to 1 γ with solutions containing as little as 150 γ per ml. of each solute.

When a large amount of one constituent is present in a mixture of solutes, it may overlap a minor constituent lying adjacent to it on a paper chromatogram irrigated with a particular solvent and preclude a reliable estimation of the minor component. The relative amounts of free amino acids in extracts of plant tissues may vary over a wide range (*25, 31, 34*). Therefore, some discrimination is necessary in the choice of chromatographic solvents employed for the separation and estimation of amino acids on paper chromatograms. In the present study three solvent systems were employed to separate seven free amino acids in orange juice. Asparagine, glutamine, histidine, cysteine, glutathione, and other minor constituents present in orange juice (*32, 39, 45, 47, 49*) are separated adequately in these solvents or are present in extremely small amounts, thereby obviating their interference with the quantitative estimations of alanine, γ-aminobutyric acid, arginine, aspartic acid, glutamic acid, proline, and serine. Quantitative estimations of cysteine and glutathione in various citrus juices have been reported previously (*26*). The amino acid values observed in the present study of California Valencia and Washington Navel orange juices are in good agreement with those presented by Wedding and coworkers (*49, 50*).

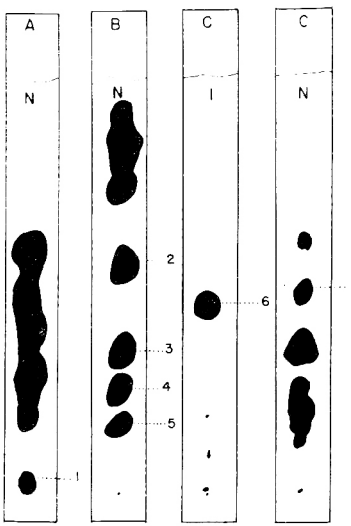

Figure 4. Tracings of typical chromatograms of orange juice

A–N. Irrigated with solvent *A*, stained with ninhydrin reagent
1. Arginine, $R_f = 0.05$
B–N. Irrigated with solvent *B*, stained with ninhydrin reagent
2. Alanine, $R_f = 0.55$
3. Serine, $R_f = 0.34$
4. Glutamic acid, $R_f = 0.26$
5. Aspartic acid, $R_f = 0.16$
C–I. Irrigated with solvent *C*, stained with isatin reagent
6. Proline, $R_f = 0.43$
C–N. Irrigated with solvent *C*, stained with ninhydrin reagent
7. γ-Aminobutyric acid, $R_f = 0.53$

MATERIALS AND APPARATUS

Filter Paper. Sheets of Schleicher & Schuell No. 507 filter paper, 2.7 × 21.5 cm., were cut from commercially available large

sheets (58 × 58 cm.) on a paper cutter with the aid of a mimeographed stencil guide similar to that described previously (*35, 36*). This filter paper was chosen because of its high purity (*33*), high transparency and uniformity (*33, 35*), low and relatively uniform mass gradient profile (*1*), slow solvent migration rate (*20, 21, 35*), absence of tailing and double spotting (*33*), and for the round and uniform spots (*20*) obtained.

Chromatographic Chamber. The arrangement was similar to that employed by Blatt (*2*) using American Medical Museum jars, size No. 11 (25 × 12 × 25 cm.). A soft rubber gasket was mounted with rubber cement on the underside of the glass cover. The hole in the cover was filled with paraffin wax. Lead weights were placed on the cover during irrigation to ensure a hermetic seal. The paper strips were mounted with self-adhesive cellophane tape on 1 × 9 × 1/16 inch slotted bars of stainless steel. The bars were supported by a stainless steel rack as shown in Figures 1 and 2. A borosilicate glass grid, immersed in the solvent at the bottom of the jar, prevented adjacent strips from coming in contact with each other during irrigation. The solvent was placed in the bottom of the jar about 2 hours prior to irrigation of the paper strips.

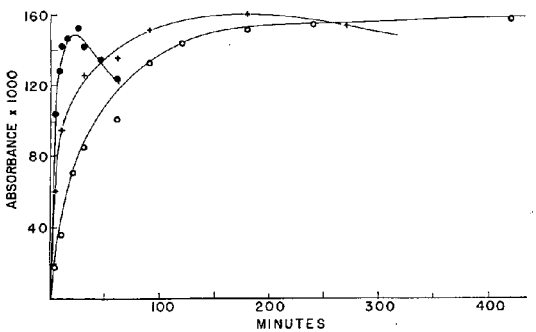

Figure 5. Effect of heating time and temperature on color intensity obtained with ninhydrin reagent and DL-alanine

S. & S. No. 507 filter paper, 2-μl. aliquots of DL-alanine solution containing total of 0.04 γ of alanine

● 55° C.
+ 44° C.
○ 30° C.

Photometer. A Lumetron photometer (Model 402 EF), equipped with a Lumetron B580 (yellow-green) optical filter, was employed for the photometric estimation of total density of the spots on both ninhydrin- and isatin-stained chromatograms. The galvanometer sensitivity was adjusted to 2 divisions for a 1% change in light transmittance, and to read 100% transmittance on a blank strip which had been treated in a manner identical to that of the standard and sample chromatograms. On occasions when excessive background coloration was observed on strips irrigated with solvent *B* (described below), the photometer was adjusted to read 100% transmittance on blank areas adjacent to the spots on each chromatogram. Increasing the transparency of stained paper chromatograms by impregnation with various materials (*19*) having refractive indices close to that of cellulose was found to be an unnecessary complication, because light scattering may also be minimized by placing the paper strips close to the photocell.

Paper Strip Adapter for Photometer. The paper strip adapter employed previously (*26, 35, 36*) for direct photometric estimation of ninhydrin-stained spots on filter paper chromatograms necessitated the removal of the complete device between each reading. Because small differences in the position of the filter paper strip may cause significant variations in light transmission, great care was required to reposition the adapter between successive photometric measurements. The device shown in Figure 3 permits reproducible, positive positioning of the filter paper strips. The base plate, *D*, conforms identically with the dimensions of the sample chamber and is bolted to its floor. The turret, *B*, is securely mounted in pivot d_3 and recess d_1. The turret has five equidistant slits, b_2, to accommodate spots of varying sizes and shapes. The three circular slits have diameters of 0.250, 0.375, and 0.500 inch, and the two elliptical slits have dimensions of 0.250 × 0.375 and 0.375 × 0.500 inch. A spring latch, d_2, in base plate recess, d_1, and the notches, b_3, serve to hold the turret and, hence, the slits in alignment. The concave inner side of strip holder *E* is recessed 0.010 inch, e_3, to accommodate a 21-mm. width of filter paper. The strip holder is locked by inserting pin e_1 in notch e_2.

The geared post, b_1, mounted on the inner concave wall of turret *B* is mechanically coupled to a gear located on the floor and between the two walls of the turret. When the heavy brass paper strip holder, *E*, is placed between the two walls of the turret so that the top of the paper strip holder is flush with the inner wall of the turret, the gear between the turret walls engages the teeth, e_4, of the paper strip holder. Rotation of geared post b_1 causes the paper strip holder to rotate and permits any spot on the filter paper to be aligned with the light beam and the appropriate juxtapositioned slits. The positioning of the spot in the center of the slit is aided by periscope *A* mounted in slot c_1 on bracket *C*. The periscope, supported by shelf a_1, is moved to the

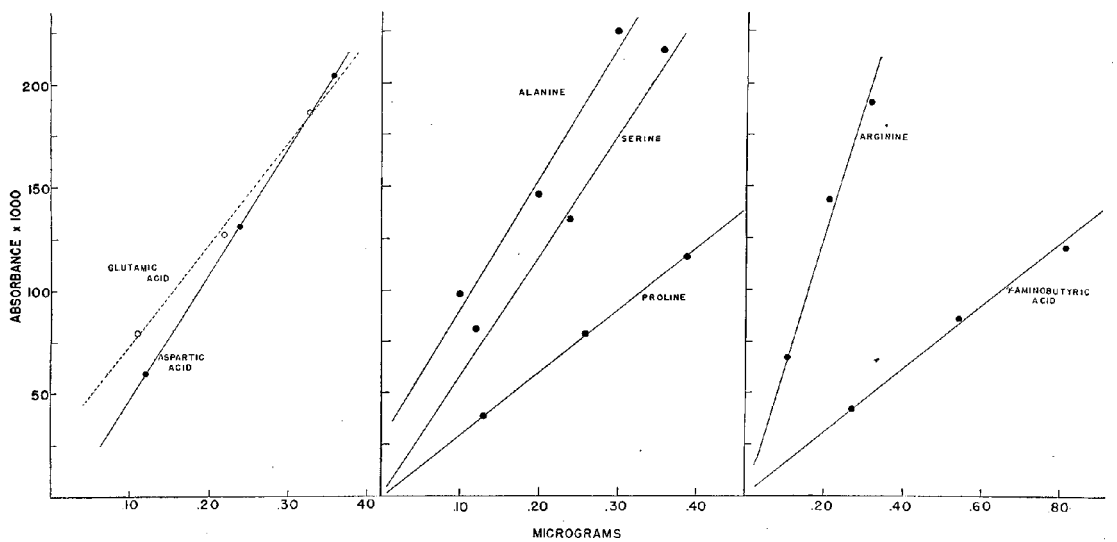

Figure 6. Representative standard curves for seven amino acids

Table I. Typical Estimation of Proline in Filtered Juice of California Valencia Oranges

Material	Concentration, Mg./Ml.	Volume Taken[a], Ml. × 10^{-4}	Absorbance × 1000						Proline Present, γ/Strip	Proline Found in Original Juice, Mg./100 Ml.
Proline standard	1.30	1.0	46	22	36	46	18	Av. 34	0.130	
		2.0	79	72	119	59	92	84	0.260	
		3.0	147	134	155	149	122	141	0.390	
Orange juice[b]		1.0	71	97	81	78	113	88	0.262[c]	105

[a] In aliquots of 5 × 10^{-3} ml. on S. & S. No. 507 filter paper; irrigated with solvent *C*.
[b] Concentrated 2.5 times; see text.
[c] Interpolated from standard curve.

Table II. Reproducibility in Estimation of Four Amino Acids in Filtered California Valencia Orange Juice[a]

Amino Acid	Found, Mg./100 Ml.[b]						Std. Dev., σ,[d] Mg./100 Ml.	Coefficient of Variation, V[e], %
						Av.[c]		
Alanine	18	29	25	25	20	23	3.0	13
Glutamic acid	26	35	30	26	29	29	2.6	9
Serine	33	34	30	26	37	32	2.9	9
Aspartic acid	51	59	58	54	59	56	2.9	5
							Av.	9

[a] Juice had Brix of 11.2 and 1.28% acid calculated as citric acid.
[b] Each value represents average of five replicate chromatograms.
[c] Represents average of 25 replicate chromatograms evaluated against five standard curves prepared on successive days. Solvent *B* and S. & S. No. 507 filter paper used.
[d] $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$
[e] $V = \frac{\sigma \times 100}{\text{av.}}$

side of the bracket, *C*, out of the incident light beam during photometric measurements. The strip holder is readily removed from between the turret walls by rotating geared post b_1 until one side of the strip holder is exposed enough to permit it to be lifted freely. All parts were anodized or painted black subsequent to taking the photographs shown. The adapter is mounted in the sample chamber of the photometer with the convex outer wall of the turret adjacent to the photocell in order to minimize light scattering and loss in sensitivity.

Solvents. Solvent *A*, 2,4,6-collidine–2,4-lutidine–water. Prepare by mixing 100 ml. each of collidine and lutidine and 92.3 ml. of distilled water at 24° C.

Solvent *B*, phenol–ammonia–water. Prepare as described by Rockland and Underwood (*38*).

Solvent *C*, *tert*-butyl alcohol–formic acid–water (69.5 to 1.0 to 29.5) (*38*).

Staining Reagents. Ninhydrin, 0.5%. Dissolve ninhydrin in a mixture of 10.0 ml. of acetic acid, 20 ml. of pyridine, 30 ml. of methyl Cellosolve, and 40 ml. of *tert*-butyl alcohol (*46*).

Isatin, 0.5%. Dissolve isatin in the same mixture employed for ninhydrin.

GENERAL PROCEDURE

Standard Solution. The standard stock solution contained 2.00 γ per ml. each of alanine, arginine (as the monohydrochloride), aspartic acid, glutamic acid, proline, and serine, and also 5.00 mg. per ml. of γ-aminobutyric acid, 25 mg. per ml. each of glucose and fructose, 50 mg. per ml. of sucrose, and 10 mg. per ml. of citric acid. The sugars and citric acid were included to minimize differences between the standard and samples in terms of the size, shape, and R_f values of the amino acid spots on paper chromatograms.

Sample Preparation. Pectin and insoluble solids were removed from each orange juice sample by adding 60 ml. of acetone to a 50.0-ml. aliquot of juice. The mixture was stirred thoroughly and allowed to stand for 10 minutes before filtering through a single 5.5-cm. sheet of S. & S. No. 507 filter paper on a Büchner funnel. The filter cake and original container were rinsed quantitatively with five 10-ml. portions of 50% aqueous acetone. The sparkling, clear filtrate was evaporated to approximately 20 ml. with a water aspirator and to a final volume of about 15 ml. with a high vacuum pump protected by traps cooled with alcohol and dry ice. The addition of a few boiling chips facilitated evaporation without excessive bumping. The concentrated juice was transferred quantitatively to a glass-stoppered graduated cylinder and made up to 20.0 ml. with distilled water.

Preparation of Chromatograms. Five replicate chromatograms were prepared for each of three levels of standard and one level of sample. The solutions were applied with a Gilmont ultramicro buret (0.01-ml. capacity) at the center and 2 cm. from the bottom of the paper strips in aliquots of 5 × 10^{-5} ml. Increasing amino acid standard levels were obtained by multiple spotting. About 30 seconds was required for the spots to dry between successive applications. Three solvent systems were required to effect satisfactory separations of the amino acids as shown in Figure 4.

Approximately 250 ml. of solvent was used in each jar. The solvent front was allowed to ascend the paper strips to within 0.5 cm. of the supporting bar or about 15 cm. from the origin. Solvent *A* separated arginine from all the major nitrogenous constituents in orange juice. Irrigation required 7 to 8 hours at a constant temperature of about 25° C. After removal from the irrigation chamber, the chromatograms were air dried for about 12 hours at room temperature before staining with ninhydrin reagent. Solvent *B* effected complete separation of aspartic acid, glutamic acid, serine, and alanine. Irrigation required 8 to 10 hours at 20° to 25° C. It was shown previously (*38*) that R_f values are not affected significantly by temperature variations within this range. Chromatograms were air dried at room temperature for about 12 hours before staining with ninhydrin reagent. Failure to effect complete removal of solvent resulted in increased background coloration and decreased precision in the estimation of alanine, because it lies in the upper portion of the chromatogram which tends to have a stronger background coloration. Shorter drying periods may be employed if a warm air stream is directed at the chromatograms. However, excessive heat may cause appreciable loss of amino acids, especially when phenolic solvents are employed (*8*, *16*, *17*, *28*).

Both proline and γ-aminobutyric acid are well separated from all other amino acids in citrus juices, although they are not completely separated from each other by solvent *C*. However, the yellow color given by proline with ninhydrin reagent does not interfere with the photometric estimation of the purple color given by γ-aminobutyric acid, and the latter does not yield any color with isatin reagent. Proline is estimated on separate chromatograms, yielding a brilliant blue color on a yellow background with the isatin reagent. Irrigation of the chromatograms required about 20 to 24 hours at a constant temperature of about 20° C. With solvent *C* small temperature fluctuations may affect R_f values significantly (*38*) and cause the volatile solvent vapors to condense on the metal supporting bars and to descend onto the paper strips. Chromatograms irrigated with solvent *C* were air dried for 30 to 60 minutes at room temperature before applying the staining reagents.

Staining Chromatograms. Wellington has reported (*51*, *52*) that maximum color development of amino acid spots on paper chromatograms with ninhydrin reagent was obtained at 20° C. and 35 to 40% relative humidity. Toennies and Kolb found that the presence of water in the reagent reduced the rate of color development (*44*). However, it has also been reported that maximum color development was obtained in a moist atmosphere (*33*, *48*). Using the reagent and conditions described herein, the present authors have obtained greater color development at 45° C. in a dry oven (below 25% relative humidity) than in a humid oven (80 to 90% relative humidity). Numerous other studies (*3*, *14*, *23*, *24*, *30*, *33*, *40*, *51*, *52*) concerned with the development of color on ninhydrin-treated amino acid chromatograms suggest that color development is a function of a large number of variables, including the type of filter paper employed, the character of the buffer or other additive present in the filter paper and/or solvent, the nature and the amount of residual solvent in the paper, the amount and relative reactivities of the different amino acids on the chromatograms, the composition and ninhydrin content of the staining solution, reaction time

and temperature, and the relative humidity or moisture content of the surrounding atmosphere. Under carefully standardized conditions, satisfactory reproducibility can probably be obtained, although the ninhydrin color reaction may not be necessarily optimal.

The dip technique (*47*) was employed in the present study because this procedure is convenient for small-scale chromatograms and permits a uniform application of staining reagents. Ninhydrin reagent was used for staining all the amino acids except proline, for which isatin reagent was used.

Figure 5 shows that the rate of color development found for alanine solutions on S. & S. No. 507 filter paper strips increased with increasing temperature. The same maximum color intensity was observed at 30°, 44°, and 55° C. However, the fleeting maximum observed at the highest temperature and the slow development of the maximum color at the lowest were not considered favorable. In addition, the highest temperature tended to increase the background coloration. Therefore, the following conditions were used with ninhydrin-stained amino acids (except γ-aminobutyric acid).

Strips were air dried under ambient conditions (20° to 25° C., 30 to 50% relative humidity) for 10 minutes, followed by heating for 60 minutes at 45° C. in a dark oven. These conditions yielded optimum color development and maximum color stability at 45° C. for 2 to 3 hours and at room temperature for at least 10 hours.

γ-Aminobutyric acid required heating for 20 minutes at 60° C. for optimum color development with ninhydrin reagent. The lower sensitivity of γ-aminobutyric acid to ninhydrin reagent is illustrated in the standard curve shown in Figure 6.

Strips being assayed for proline were immersed for 1 to 2 seconds in the isatin reagent, drained and dried for 5 minutes in air, and then heated for 10 minutes at 95° C. The brilliant blue color obtained with proline was found to be relatively unstable. In order to minimize variations in replicate strips, it was necessary to handle each set of eight strips as a unit, and to estimate the spot densities immediately after heating. Because less than 5 minutes was required to estimate the color density of a set of eight strips, a routine sequence of operations was possible without unnecessary loss of time.

Photometric Estimation. New standard curves were prepared for each series of assays because of anomalous variations in the background color after treatment with ninhydrin. Representative standard curves for seven amino acids, prepared from data analogous to that presented in Table I, are shown in Figure 6. Essentially linear standard curves were obtained in the range from 0.1 to 0.4 γ for six amino acids and from 0.2 to 0.8 γ for γ-aminobutyric acid.

An example of the data obtained in a typical estimation of proline in orange juice is presented in Table I. Table II illustrates the reproducibility obtained in the estimations of analine, glutamic acid, aspartic acid, and serine in a sample of filtered California Valencia orange juice. An evaluation of the precision obtained in the estimation of seven amino acids in the filtered juices of California Valencia and Washington Navel oranges is presented in Table III. The standard error of the means, calculated for five replicate paper strips, ranged from 2.3 to 13.1% of the arithmetic means and averaged 8.5 and 8.7% for the seven amino acids in California Valencia and Washington Navel orange juices, respectively.

DISCUSSION

The direct estimation of total spot density on small-scale paper chromatograms has a number of advantages over other paper chromatographic techniques which have been suggested for the estimation of amino acids. Aside from its speed and convenience, the method is not affected significantly by irregularities in the size or shape of the spots nor the distribution of color within the spots. Figure 7 indicates that, within the optimum analytical range, no significant change in photometric total spot density measurements was observed as the spot size increased at unit concentration of DL-alanine. The data employed to construct this figure were obtained by spotting strips of S. & S. No. 507 filter paper with increasing single aliquots of decreasing concentrations of alanine solutions, adjusted so that each spot contained 0.05 γ of alanine spread uniformly over the wetted area. The distribution of alanine on the filter paper ranged from 25.0 to 0.25 γ per sq. cm. After being dried, the paper strips were treated with ninhydrin solution and the colors were developed at 45° C. for 1 hour. The total density of each spot was determined as described previously. A 0.375-inch aperture was employed in order to enclose the largest spot and leave a small white margin.

Figure 7 shows that the total color intensity increased rapidly as the alanine content decreased to about 50 γ per ml., or 0.5 γ per sq. cm. of filter paper. Moreover, when the total volume of alanine solution applied to the filter paper was greater than 100×10^{-3} ml., or below 0.5 γ of alanine per sq. cm. of filter paper, the size of the spot did not affect the measured absorbance. Because the color of ninhydrin-stained amino acid spots is not distributed uniformly on actual chromatograms, it was concluded that, with the exception of α-aminobutyric acid, less than 0.5 γ of each amino acid should be employed per strip in order to minimize variations in photometric readings due to irregularities in the size or shape of the spots.

The present procedure was designed so that the final colored spot areas varied between 0.5 and 1.2 sq. cm. and contained from 0.2 to 0.4 γ of amino acid per square centimeter of filter paper. Compared with the other amino acids, γ-aminobutyric acid yields a ninhydrin reaction product of considerably lower color density. Figure 6 shows that from two to seven times as much γ-aminobutyric acid is required to give a spot density equal to that observed for the other six amino acids.

The relatively small photocell in the Lumetron 402 EF photometer precludes the use of this instrument for the estimation of total density in spots having a diameter greater than about 5/8 inch. However, apparatus similar to those designed by Sendroy and Cecchini (*43*) and Polson and coworkers (*30*) might be employed for larger spots such as are obtained on conventional types of paper chromatograms. For maximum sensitivity the aperture should be slightly larger than the spot. With the photometer, optical filter, the filter paper employed in the present studies, a circular aperture having a diameter of less than 0.25 inch did not transmit sufficient light to permit the instrument to be balanced satisfactorily.

A circular aperture having a 0.375-inch diameter was found to be most generally applicable.

Values found in the present study and by Wedding and coworkers (*49*, *50*) for seven free amino acids in California

Table III. Precision in Estimation of Seven Free Amino Acids in Filtered Juices of California Valencia and Washington Navel Oranges

	California Valencia				Washington Navel			
Amino Acid	Replicate strips	Range, mg./100 ml.	Av., mg./100 ml.	Std. dev., mg./100 ml.	Replicate strips	Range, mg./100 ml.	Av., mg./100 ml.	Std. dev., mg./100 ml.
Serine	10	14 to 25	19	3	9	17 to 56	33	11
Alanine	10	14 to 36	24	6	10	19 to 48	34	11
Glutamic acid	10	14 to 30	20	4	10	29 to 61	37	12
γ-Aminobutyric acid	12	16 to 41	30	9	12	14 to 60	33	11
Aspartic acid	10	24 to 61	38	13	12	38 to 88	65	17
Arginine	12	92 to 144	115	16	12	121 to 166	144	10
Proline	12	89 to 174	126	49	12	172 to 248	235	42

Table IV. Some Free Amino Acids in California Valencia and Washington Navel Orange Juices

Amino Acid	California Valencia, Mg./100 Ml.			Washington Navel, Mg./100 Ml.		
	Authors[a]	Wedding, Horspool (*49*)[b]	Wedding, Sinclair (*50*)[c]	Authors[d]	Wedding, Horspool (*49*)[b]	Wedding, Sinclair (*50*)[c]
Alanine	19 to 25	3 to 22	13	18	5 to 26	18
Serine	14 to 36	5 to 37	4	23	9 to 33	30
Glutamic acid	19 to 34	11 to 49	71	19	6 to 31	21
Aspartic acid	32 to 64	8 to 115	94	44	7 to 89	41
γ-Aminobutyric acid	30 to 73	6 to 254	9	38	4 to 33	21
Arginine	90 to 150	27 to 118[e]	119[e]	82	23 to 98[e]	73[e]
Proline	126 to 250	49 to 164	69	295	6 to 115	103

[a] Range of values found for 20 miscellaneous samples of fresh filtered juice.
[b] Range for samples of varying maturity.
[c] Average of three samples of the juice from mature fruit, calculated from data presented by Wedding & Sinclair (*50*).
[d] Average of two estimations on single sample.
[e] Reported as lysine; see text.

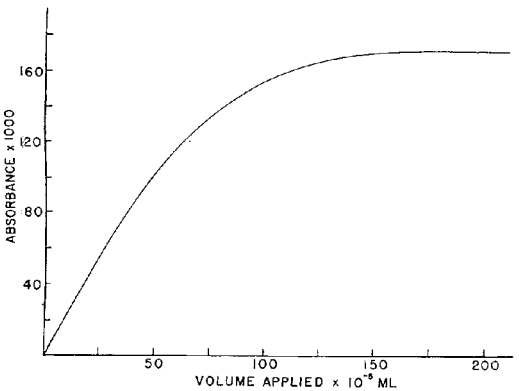

Figure 7. Variation of spot color, measured photometrically, with increasing volumes and corresponding spot sizes of solutions containing identical total amounts of DL-alanine

Valencia and Washington Navel orange juices are summarized in Table IV. Most of the data are in reasonably good agreement considering the high variability that might be expected in natural products of this kind. Wedding and Horspool reported the presence of relatively large amounts of lysine and the absence of arginine in both Valencia and Navel orange juices. The presence of arginine and traces of lysine in orange juice has been reported previously by several laboratories (*32, 39, 45, 47*). The present authors have obtained positive Sakaguchi color tests for arginine in fresh juice as well as at the appropriate position on paper chromatograms of filtered orange juice. It would appear that orange juice contains traces of lysine and variable but significantly higher levels of arginine. Because the present procedure does not separate or differentiate arginine and lysine, the values presented for arginine in columns 2 and 5 in Table IV represent the sum of arginine and lysine. It is suggested that the values presented by Wedding and coworkers for lysine may also represent the sum of these two constituents.

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RECEIVED for review May 29, 1956. Accepted August 6, 1956. The mention of special instruments or materials does not imply endorsement by the Department of Agriculture over others of similar nature.

Gas Chromatographic Determination of Some Hydrocarbons in Cigarette Smoke

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This paper concerns the results of experiments designed to study the effectiveness of gas chromatographic methods for the separation and analysis of some components of the gaseous phase of cigarette smoke. By using a column containing silica gel and employing helium as the carrier gas, it was possible to determine seven hydrocarbons in a 10-ml. sample of smoke from which the particulate matter had been removed by a special filter. The gas chromatographic method described is a simple and effective means of determining low concentrations of the compounds studied.

THE work described in this paper was undertaken with the general object of studying the effectiveness of gas chromatography for the separation and analysis of some components of cigarette smoke. Preliminary experiments with the method indicated that several types of components could be detected in the gaseous phase of cigarette smoke. Included among these were seven light hydrocarbon gases. Although most of these compounds had been reported present in cigarette smoke, quantitative information was not available (*5*). Therefore, the study was concentrated on the quantitative determination of these hydrocarbons found in cigarette smoke produced under varying smoking conditions. Quantitative results obtained by means of infrared absorption have been reported since this work was completed (*13*). Considering the differences in the samples used, the results agree as well as can be expected with those reported here.

Although gas chromatographic methods are relatively new, their remarkable effectiveness in the separation of gases and volatile liquids has been amply demonstrated. The number of recent papers devoted to investigations in this field is evidence that the use of gas chromatography is rapidly becoming widespread. In addition to general papers on the subject (*2*, *6–12*, *15*, *16*), gas chromatographic methods have been applied to the analyses of small quantities of materials produced during kinetic studies (*3*), fluorinated compounds (*4*), hydrocarbons in automobile exhaust gases (*14*), and mixtures of methyl ketones (*1*).

APPARATUS AND MATERIALS

Gas Chromatographic Apparatus. The gas chromatographic apparatus was essentially the same as that previously described (*15*), except as noted.

Helium was used as the carrier gas, with a flow rate of 50 ml. per minute. The thermal conductivity cell, used as the detector, was made by the Gow-Mac Instrument Co. to the specifications submitted by the authors (*15*). The output of the thermal conductivity cell was recorded by a recording millivoltmeter (Leeds & Northrup Co.) with a range of 0 to 1 mv.

The column and sample container are shown in Figure 1. The portion of the column packed with silica gel was 130 cm. long and 0.55 cm. in inside diameter. The shorter portion, containing Ascarite for removal of carbon dioxide and water, was 15 cm. long and 0.7 cm. in inside diameter. The two-way stopcock between the two sections prevented air from reaching the silica gel while the sample containers were being changed. The column was used at 25° ± 2° C. The gas sample container was constructed as shown in Figure 1. Each of the two sections of glass tubing between the two stopcocks had a volume of 10 ml. The sample was collected in one of these, and the other served as a bypass for the carrier gas until the chromatographic system was ready for a run.

The adsorbent used for packing the column was a type of silica gel having a low density and large pore size (Davison Chemical Co., Grade 70). Particles 30 to 50 mesh in size were packed into the column and held in place by glass wool. Helium was passed through the column for about 3 days before it was used for quantitative work. Columns packed with oven-dried (130° C.) silica gel required less conditioning to produce a stable background response from the thermal conductivity cell. However, it was found that a sharper separation of propylene from butane could be obtained when the silica gel column was conditioned at room temperature. This difference is possibly related to the amount of moisture remaining in the adsorbent after conditioning.

Calibration of Apparatus. Calibrating mixtures containing known amounts of hydrocarbons in nitrogen were prepared with the aid of the apparatus shown in Figure 2.

The large spherical vessel had a volume of about 1 liter. The volumes of the smaller containers were chosen so as to produce desired concentrations of the hydrocarbons being added. The sample chamber of each of seven such vessels (only two of which are shown in the figure) was filled with one of the seven hydrocarbons studied. These were connected in series with stopcocks turned to provide an open passage through the bypass side of each vessel. One terminal container was attached to the spherical vessel and the other to a source of nitrogen. The system was evacuated and the stopcock between the spherical vessel and the

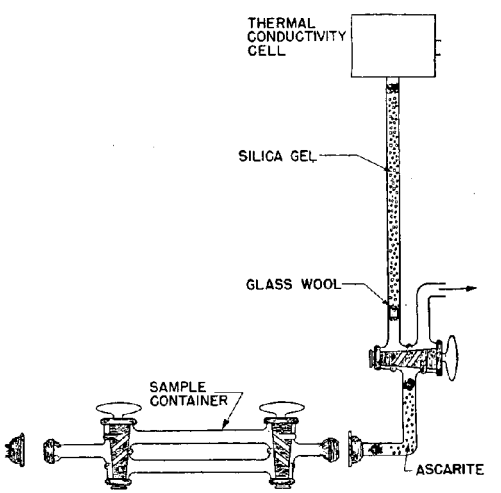

Figure 1. Adsorption column and sample container

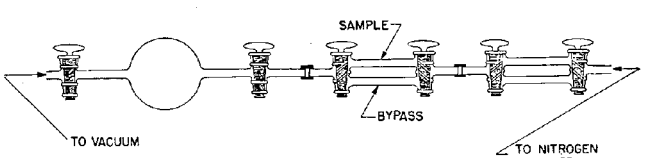

Figure 2. Apparatus for preparing calibration mixtures

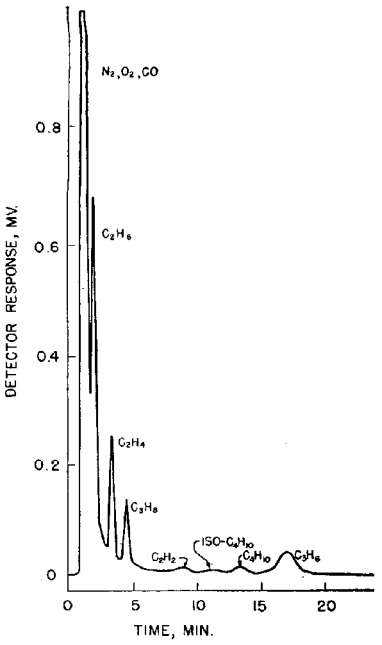

Figure 3. Elution of smoke from silica gel by helium

vacuum pump was closed. The stopcocks on the small containers were then turned to provide an open path through the sample chambers. Nitrogen was allowed to sweep the hydrocarbon samples into the spherical vessel and fill it to atmospheric pressure. The mixture was made homogeneous by adding mercury to the spherical vessel and shaking it.

Apparatus for Collecting Gas Phase of Cigarette Smoke. The gas samples were obtained from cigarettes smoked in a manner that simulated normal smoking conditions. This was accomplished by employing a smoke collection train consisting of an automatic cigarette smoking machine, a specially designed filter for removing the liquid-solid phase of the smoke, and a gas sample container for collecting a portion of the smoke gases. The cigarette smoking machine was discussed in an earlier paper (*17*).

In order to vary the duration of the 35-ml. puff taken by the smoking machine, a rubber tube was inserted into the system at the bottom of the buret. Changing the degree of constriction at this point by means of a screw clamp made it possible to adjust the duration of the puff from 1.3 to 3 seconds.

The filter for removing the liquid-solid phase of the smoke consisted of a disk of Cambridge filter material 1 mm. thick and 35 mm. in diameter sealed between two glass funnels directly behind the cigarette. The total volume within the two funnels was approximately 25 ml. The gas sample container previously described was connected to the smoking apparatus immediately following the filter.

Selection and Smoking of Cigarettes. Three brands of unfiltered, king-size cigarettes manufactured and distributed in the United States were employed in all of the smoking experiments. The cigarettes had a moisture content of 12 ± 0.5% and weighed within ±2% of their average weight. They were stored and smoked at a relative humidity of 55 to 60% and a temperature of 75° F.

The smoke collection train, consisting of cigarette, Cambridge filter, and gas sample container, was assembled and the smoking machine was standardized for a 35-ml. puff of the desired duration. This was one of the three puff durations studied—namely, 1.3, 2.0, or 3.0 seconds. The interval between puffs was adjusted to 1 minute. After the device was standardized, the puff counter was adjusted to zero.

Except for one series of experiments, only one sample was obtained from each cigarette. This material was collected during

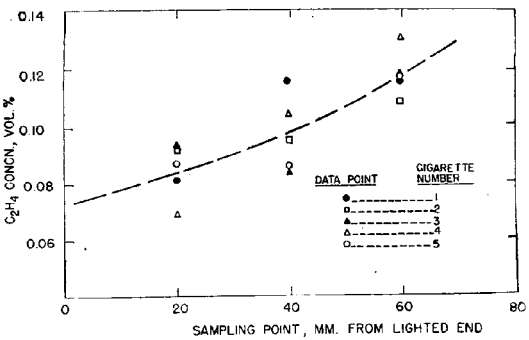

Figure 4. Relation of ethylene concentration in smoke to cigarette butt length

the puff that caused the burning zone to move past a pencil mark initially 40 mm. from the lighted end. In the one exception, three samples were collected corresponding to points initially 20, 40, and 60 mm. from the lighted end.

The procedure for collection was as follows. Until the burning zone reached the point for collection, the smoke was pulled through the bypass side of the gas sample container. When it was evident that the next puff would cause the glowing coal to obliterate the pencil mark, the stopcocks on the sample container were turned to direct the gases through the sample side. At the end of this puff the stopcocks were turned to provide an open path through the bypass side again. In this way a 10-ml. sample of the gaseous phase of the smoke was collected.

Samples collected in this manner were analyzed by means of the gas chromatographic method. The filled sample container with the stopcocks turned to the bypass side was connected into the chromatographic system (see Figure 1). After all connections were made and the system was ready for a run, both stopcocks on the sample container were turned so that the carrier gas swept the sample onto the column of silica gel. The recording potentiometer automatically plotted the output of the thermal conductivity cell against time.

RESULTS

A typical curve for the elution of a smoke sample is shown in Figure 3. Tentative identification of the compounds responsible for the peaks was based on similar curves for known substances. Confirmation of the identity of each compound was obtained by collecting the material corresponding to each peak and passing it through a similar gas chromatographic system in which the column was packed with Celite 545 (Johns Manville) impregnated with hexadecane. Methane was undoubtedly present, but it was not separated from nitrogen by the silica gel column.

Quantitative results are based on calibration with known mixtures, as already described. It is evident from the curve in Figure 3 that the peak for ethane partially overlaps that due to nitrogen and other very low-boiling gases. This is also true to a lesser degree for ethylene and propane. It was to compensate for this "background" response that the calibration mixtures were prepared with nitrogen as the major component. Individual determinations of ethane, ethylene, propane, and propylene are believed to be accurate to better than ±5% of the amount found. Because of the minute amounts present, acetylene, isobutane, and butane could not be determined with sufficient precision to detect changes associated with the variables studied. The values reported for these three compounds may be in error by a factor of 2.

Samples from three brands of cigarettes were analyzed. They were smoked according to the procedure described, with a puff of 2 seconds' duration unless otherwise specified. Each value reported is the average for five runs.

The concentrations of ethane, ethylene, propane, and propylene

in smoke from the brands studied are listed in Table I. These samples were obtained after smoking to an arbitrary point 40 mm. from the lighted end. No significant difference in the three brands is indicated by these figures.

Samples collected after smoking brand A cigarettes a distance of 40 mm. with varying duration of puff were used to obtain the values in Table II. Although the 1.3-second puff seems to give lower concentrations than the standard 2.0-second puff, the differences proved to be statistically significant at the 95% confidence level for propane and propylene only. None of the differences between corresponding values for the 2.0-second puff and the 3.0-second puff were statistically significant.

Results for smoke from brand A cigarettes at 20, 40, and 60 mm. are compared in Table III. The concentrations of each of the four hydrocarbons increased with decreasing length of the cigarette butt. Statistical analysis indicated these increases to be significant at the 95% confidence level.

The data for ethylene are shown graphically in Figure 4. Corresponding graphs for the other components are similar. A smooth curve joining the averages for each of the three sampling points is somewhat convex toward the abscissa in each case. Because of the precision of the data, the deviation from linearity is not significant.

Table IV contains the calculated average concentration of each of the seven hydrocarbons studied in all of the main streams of smoke produced by smoking brand A cigarettes a distance of 60 mm. from the lighted end. These values were obtained by reading the concentration of the 30-mm. point from graphs like the one for ethylene in Figure 4. The amount of each hydrocarbon present in the smoke from a standard package of cigarettes is also listed. These figures were calculated by assuming that an average of 12 35-ml. puffs (420 ml.) is produced by smoking 60 mm. of each cigarette.

Analyses were made on smoke from brand A cigarettes that had been cut off at the 40-mm. mark and smoked to the 60-mm. mark. The results are shown in Table V along with figures obtained from similar cigarettes smoked under the same conditions all the way from the lighted end to the 60-mm. mark. Corresponding values in the two columns are not significantly different.

These data indicate that the increase in concentration of the hydrocarbon gases with decreasing butt length (see Table III) is not primarily the result of pyrolysis of accumulated tars. If this were the case, the values obtained for the short cigarettes should correspond to those obtained for the full length cigarettes at the 20-mm. mark.

The possibility that the hydrocarbon gases are adsorbed or absorbed by the tobacco and/or tar cannot be ruled out on the basis of the experiments performed. It is also possible that the decrease in pressure drop across the cigarette as its length decreases may increase the rate at which air is drawn through the cigarette during a puff. However, one would expect from the results obtained by varying the duration of puff (see Table II) that this would lead to decreasing concentrations as the smoking proceeded. It might be enlightening to be able to measure the temperature of the burning coal when each sample is taken.

Table I. Variation of Hydrocarbon Concentration with Brand of Cigarette

	Concentration, Volume %		
Hydrocarbon	Brand A	Brand B	Brand C
Ethane	0.19	0.17	0.17
Ethylene	0.097	0.093	0.097
Propane	0.057	0.057	0.054
Propylene	0.069	0.064	0.067

Table II. Variation of Concentration with Duration of Puff

	Concentration, Volume %		
Hydrocarbon	1.3-sec. puff	2.0-sec. puff	3.0-sec. puff
Ethane	0.16	0.19	0.19
Ethylene	0.084	0.097	0.090
Propane	0.051	0.057	0.059
Propylene	0.057	0.069	0.063

SUMMARY

A gas chromatographic method for quantitative determination of ethane, ethylene, acetylene, propane, propylene, isobutane, and butane in the gaseous phase of cigarette smoke was found to be remarkably effective for the separation of these components from others present in this complex mixture. It had sufficient sensitivity to enable their determination in a 10-ml. sample. Ethane, ethylene, propane, and propylene were present in amounts sufficient to enable determination of changes in their concentrations accompanying variations in the smoking procedure. The concentrations of these compounds increased as the length of the cigarette butt decreased.

Although the silica gel column described is suitable for the determination of light hydrocarbons only, the use of other column packings and conditions should enable analysis of some other components of cigarette smoke. The gas chromatographic method should also be useful for separating components present in the gaseous phase of cigarette smoke for identification by other means, particularly mass spectrometry.

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Table III. Variation of Hydrocarbon Concentration with Length from Lighted End

	Concentration, Volume %		
Hydrocarbon	20 mm.	40 mm.	60 mm.
Ethane	0.15	0.19	0.25
Ethylene	0.084	0.097	0.117
Propane	0.051	0.057	0.065
Propylene	0.059	0.069	0.076

Table IV. Average Concentration of Seven Hydrocarbons in Cigarette Smoke

Hydrocarbon	Volume %	Ml. (S.T.P.) from 20 Cigarettes
Ethane	0.2	17
Ethylene	0.1	8
Acetylene	0.01	0.8
Propane	0.06	5
Propylene	0.07	6
Isobutane	0.001	0.1
Butane	0.006	0.5

Table V. Comparison of Concentration for Full Length and Shortened Cigarettes Smoked to Same Butt Length

	Concentration, Volume %	
Hydrocarbon	Full length	40 mm. shorter
Ethane	0.25	0.22
Ethylene	0.117	0.114
Propane	0.065	0.067
Propylene	0.076	0.077

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RECEIVED for review January 9, 1956. Accepted July 6, 1956. Presented at the Tobacco Chemists' Research Conference, North Carolina State College, Raleigh, N. C., October, 6, 1955.

Titration of Copper Oxinate in Glacial Acetic Acid

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Organic precipitants are very useful for separating metal ions, but gravimetric determinations involving direct weighing of the precipitated metal chelates are often beset by difficulties, including not only the usual gravimetric tedium, but also errors arising from dubious weighing forms which exhibit uncertain hydration, decomposition, and volatility. It is thus of interest to investigate methods other than gravimetric for the measurement of these analytical precipitates. In the present study, it is shown that copper can be determined by applying nonaqueous titrimetry to the copper oxinate precipitate. Cupric ion is precipitated from aqueous solution with oxine, the copper oxinate is dissolved in glacial acetic acid, hydrogen sulfide is bubbled through the solution to precipitate copper sulfide, and finally the oxine solution is titrated with perchloric acid to a potentiometric end point. The method yields very satisfactory results.

BECAUSE organic precipitants are, in general, weak acids and/or bases, it was reasonable to investigate the application of nonaqueous titrimetry to these reagents and their metal complexes. One might hope thereby to retain the attractive features of organic precipitants in effecting separations while circumventing some of the difficulties attending their gravimetric use. Some preliminary studies along these lines with 8-quinolinol (8-hydroxyquinoline, oxine) and dimethylglyoxime have been reported by Fritz (*3*).

When oxine is dissolved in glacial acetic acid and titrated potentiometrically with a solution of perchloric acid in glacial acetic acid, an excellent end point is obtained (Figure 1). If, on the other hand, a metal oxinate rather than oxine itself is dissolved in acetic acid and titrated, the end point is very poor (Figure 1). This has proved true in the case of iron, copper, aluminum, magnesium, and a number of other metals (*4*). Studies of the extents to which the various metal oxinates dissociate in acetic acid, and the dissociation of the resultant metal acetates, would be needed to explain the difficulty completely. Presumably the following equilibria are involved:

$$M(Ox)_n + nHOAc \leftrightharpoons nHOx + M(OAc)_n$$

$$HOx + HOAc \leftrightharpoons (H_2Ox)^+ + OAc^-$$

$$M(OAc)_n \leftrightharpoons M^{+n} + nOAc^-$$

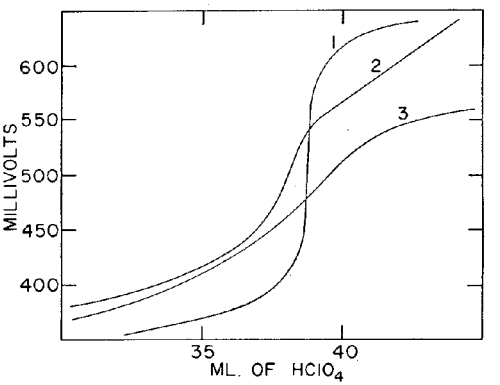

Figure 1. Titration curves of oxine and metal oxinates

1. Oxine
2. Aluminum oxinate
3. Copper oxinate

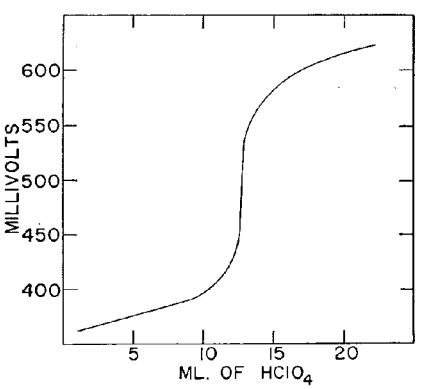

Figure 2. Titration of copper oxinate solution after precipitation of copper sulfide

Table I. Titration of Copper Oxinate with Perchloric Acid

Copper Oxinate, Mg.		Deviation	
Taken	Found	Mg.	%
226.0	225.5	0.5	0.22
254.3	255.1	0.8	0.31
260.4	260.7	0.3	0.12
314.6	312.8	1.8	0.57
324.7	324.4	0.3	0.09
370.5	369.5	1.0	0.27
373.9	374.8	0.9	0.24
389.3	392.9	3.6	0.92
401.5	401.2	0.3	0.07
473.7	471.6	2.1	0.44
497.4	496.2	1.2	0.32
504.9	505.0	0.1	0.02
523.6	519.6	4.0	0.76
536.9	536.9	0.0	0.00
544.1	539.0	5.1	0.94
553.4	550.9	2.5	0.45
594.8	591.3	3.5	0.59
743.4	743.5	0.1	0.01
872.3	867.5	4.8	0.55
881.4	881.3	0.1	0.01
891.5	886.4	5.1	0.57
	Av. deviation	1.8	0.20
	Standard deviation	2.6	

Table II. Precipitation of Copper from Aqueous Solution Followed by Nonaqueous Titration

Copper, Mg.		Deviation	
Taken	Found	Mg.	%
72.2	71.8	0.4	0.55
72.2	72.0	0.2	0.28
72.2	72.1	0.1	0.14
72.2	72.4	0.2	0.28
96.2	95.6	0.6	0.62
96.2	95.9	0.3	0.31
96.2	95.9	0.3	0.31
96.2	96.0	0.2	0.21
96.2	96.6	0.4	0.42
120.3	119.5	0.8	0.67
120.3	119.6	0.7	0.58
120.3	119.7	0.6	0.50
120.3	119.7	0.6	0.50
120.3	119.9	0.4	0.33
120.3	120.1	0.2	0.17
120.3	120.5	0.2	0.17
144.4	143.5	0.9	0.62
144.4	143.5	0.9	0.62
144.4	143.9	0.5	0.35
144.4	143.9	0.5	0.35
	Av. deviation	0.4	0.32
	Standard deviation	0.5	

The equilibrium constants that would be needed for a complete interpretation of the problem are not at hand.

A workable egress from this difficulty is applicable in cases where the metal in question forms a sulfide that is insoluble in glacial acetic acid. When hydrogen sulfide is bubbled through the solution, the difficulty clears up, as shown in the following reaction for the case of copper:

$$Cu(OAc)_2 + H_2S \rightleftharpoons CuS\downarrow + 2HOAc$$

The precipitated metal sulfide does not interfere with the potentiometric titration, and the other product is, of course, the solvent itself.

Unfortunately, copper and cadmium are the only examples from a large number of metals tested where the sulfide precipitation, the solubility of the oxinate in acetic acid, and an unambiguous formula for the oxinate are all favorable for an accurate determination. In this paper, a workable method for the determination of copper based upon the above principles is presented. Briefly summarized, the method is as follows: Cupric ion is precipitated from aqueous solution in the usual manner with oxine, and the copper oxinate is collected by filtration, washed, and then dissolved from the filter with glacial acetic acid. Hydrogen sulfide is bubbled through the acetic acid solution, and the solution is finally titrated with a standard solution of perchloric acid in glacial acetic acid.

The precipitation of copper oxinate, and the separations which can be accomplished thereby, have been thoroughly discussed (*1*). Thus it was not necessary to investigate this phase of the method, and attention was centered upon the actual determination of the copper oxinate. The titration proposed here is very much faster than drying and weighing the precipitate, and apparently at least equally reliable. It is somewhat faster and probably more accurate than the bromometric titration of oxine.

EXPERIMENTAL PROCEDURES

Apparatus and Reagents. A Beckman Model H pH meter with a glass electrode and a sleeve-type calomel electrode was used for the potentiometric titrations. The titrant was 0.1*N* perchloric acid in glacial acetic acid, prepared according to Fritz (*2*). This solution can be standardized against potassium acid phthalate; the same normality was obtained in this way as with pure oxine as the standard. All materials were reagent grade. Copper oxinate was prepared according to directions by Flagg (*1*). Standard copper solutions were prepared from electrolytic copper foil in the approved manner. A small lecture cylinder of hydrogen sulfide is a convenient source of this gas, although a Kipp generator can be used.

Titrations of Pure Copper Oxinate. To test the reliability of the titration itself, apart from possible errors in the precipitation of copper as oxinate, accurately weighed portions of pure copper oxinate were dissolved in glacial acetic acid. Very roughly, 50 ml. of acetic acid is required to dissolve each 100 mg. of copper oxinate. The range studied was from about 200 to 900 mg. of copper oxinate. Hydrogen sulfide was bubbled through the solution for about 3 minutes. The precipitated cupric sulfide was coarsely granular and settled to the bottom of the beaker. The supernatant solution was yellow when the precipitation of copper was complete; otherwise green. It was not necessary to separate the precipitate from the solution, nor did excess hydrogen sulfide interfere in any way. A few drops of acetic anhydride were added to the solution just prior to the titration, to eliminate water. Traces of water are not objectionable, but larger amounts make the end point less sharp. The solution was titrated potentiometrically with the standard perchloric acid solution.

The end points in these titrations were rather good, as may be seen from the typical titration curve shown in Figure 2. Volumes of titrant were calculated by the differential method (*5*). The results of these titrations are summarized in Table I, where it may be seen that the titrations are apparently free of any important error.

Combined Precipitation and Titration. To establish the over-all reliability of the method combining separation of copper from aqueous solution as the oxinate with the nonaqueous titration, a second series of experiments was performed. The copper was precipitated from aliquots of a standard cupric nitrate solution according to directions given by Flagg (*1*). The precipitates were collected, washed, and dissolved in glacial acetic acid. Copper was precipitated with hydrogen sulfide, and the titrations were performed as in the first series. The quantities of copper ranged from about 70 to 150 mg. The results of this study are presented in Table II, where again it is indicated that the method is sound.

ACKNOWLEDGMENT

Charles H. Hill is indebted to the New York Community Trust for a fellowship during the period when the work was performed.

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High Frequency Titrations of Some Substituted Anilines in Glacial Acetic Acid

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A high frequency titration apparatus operating at 130 megacycles was used to titrate aniline and nine substituted anilines dissolved in acetic acid. The method was successfully used to titrate each component of mixtures of six pairs of substituted anilines, but conductometric titration with a commercial-type instrument did not give resolution for two of these mixtures.

DURING the past five years titrations in nonaqueous media have received considerable attention. The booklet by Fritz (*2*) and reviews by Riddick (*10-12*) list a very complete and extensive bibliography of published determinations. A review slanted particularly to pharmaceutical applications was published by Pifer, Wollish, and Schmall (*9*). One pertinent problem of concern is the detection of the equivalence point of titrations performed in nonaqueous media.

The purpose of the present investigation was to initiate a systematic study on the possible application of high frequency titrimetry to weak acids and bases in nonaqueous solvents. Aniline and substituted anilines were titrated in acetic acid with acetic acid solutions of perchloric acid in a titrator operating at a frequency of 130 Mc. Aniline and nine substituted anilines were titrated individually.

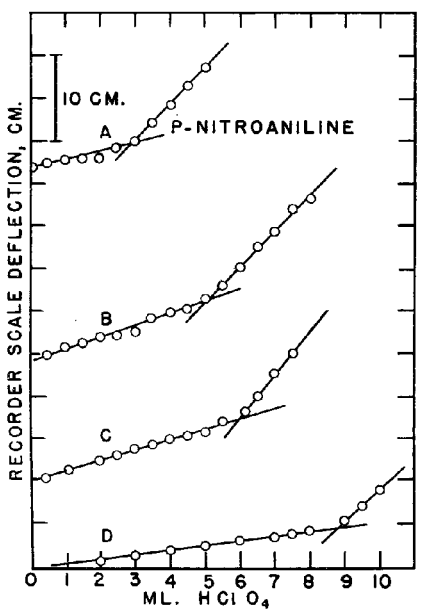

Figure 1. Effect of concentration on high frequency titrations of *p*-nitroaniline in glacial acetic acid

A. 2.56 × 10^{-3} *M*
B. 1.18 × 10^{-2} *M*
C. 1.22 × 10^{-2} *M*
D. 2.08 × 10^{-2} *M*

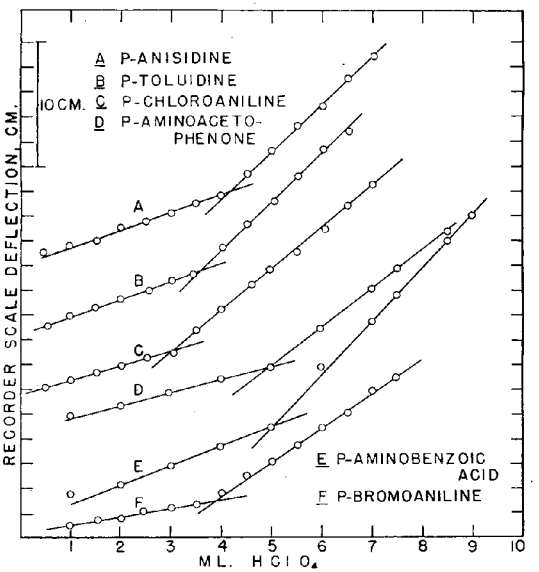

Figure 2. High frequency titrations of substituted anilines in glacial acetic acid

A. *p*-CH_3O, 9.04 × 10^{-3} *M*
B. *p*-CH_3, 8.32 × 10^{-3} *M*
C. *p*-Cl, 6.70 × 10^{-3} *M*
D. *p*-$COCH_3$, 1.18 × 10^{-2} *M*
E. *p*-CO_2H, 1.2 × 10^{-2} *M*
F. *p*-Br, 8.54 × 10^{-3} *M*

Individual compounds were determined successfully in six samples consisting of pairs of substituted anilines.

High frequency titrations of bases in acetic acid (*5*, *13*), organic bases and amino acids in benzene-methanol–acetic acid (*8*), organic acids in benzene-methanol mixtures (*4*), acids in dimethylformamide (*1*), and organic acids in a variety of solvents (*7*) have thus far been reported. In one of these studies (*13*) aniline and *p*-toluidine were titrated, but attempts to titrate *p*- or *o*-nitroaniline were unsuccessful. Very recently a successful titration of *p*-nitroaniline was reported (*7*).

APPARATUS

The high frequency titrator was identical with that described by Johnson and Timnick (*6*). The sensitivity of this instrument operating at a frequency of 130 Mc. was satisfactory for this study.

Titrations were carried out in a thin-walled cell constructed from a 250-ml. polyethylene bottle. In all titrations the liquid level in the cell was above the top of a grounded ring in the cell assembly.

Solutions were stirred during titrations with a motor-driven paddle stirrer. Calibrated burets were used.

The Sargent Model XXI Polarograph was used to measure the

change in grid current in the oscillator circuit (*6*). Direct reading of the recorder scale provided a measure of the grid current after each addition of titrant. A span potential of 1 volt and sensitivities of 0.040 or 0.060 μa. per mm. of scale deflection were used in all titrations, because, under these conditions, the change in current during the course of a titration covered an appreciable portion of the 280-mm. recorder scale.

Potentiometric titrations were carried out with a Fisher Titrimeter, using a glass indicator electrode and a fiber-type saturated calomel reference electrode, in a 150-ml. beaker containing the aniline and 100 ml. of acetic acid.

Conductometric titrations were performed in a 250-ml. beaker, with 100 ml. of solution, employing a dipping cell with a cell constant of 0.1. Universal Products conductivity bridges, Models RC-16 and RCM15, were used. Sharper bridge balance was obtained at 60 than at 1000 c.p.s. Attempts to sharpen the end point with the aid of the decade capacitor, Model DK-2, were unsuccessful.

REAGENTS

Glacial acetic acid, acetic anhydride, and perchloric acid, 70 to 73%, conforming to A.C.S. specifications.

Acid potassium phthalate, primary standard grade.

Aniline, *p*-chloroaniline, *p*-bromoaniline, *p*-aminoacetophenone, *p*-aminobenzoic acid, *p*-nitroaniline, *o*-nitroaniline, *m*-nitroaniline, *p*-anisidine, and *p*-aminophenol were obtained from the Eastman Kodak Co. in the highest purity available. *p*-Toluidine was obtained from Merck and Co., Inc., and 2,4-dinitroaniline was obtained from the Paragon Testing Laboratories. All solid anilines were recrystallized until their melting points were constant within 1°. The aniline was purified by simple distillation.

Perchloric acid, 0.3*N* in acetic acid, plus acetic anhydride to react with water, was standardized with potassium acid phthalate to the crystal violet end point.

Table I. Titrations of Organic Bases

Base	$HClO_4$ Used, Ml.	Base Taken, Gram	Base Found, Gram	Purity of Base, %
Aniline	3.44	0.08769	0.0869	99.1
	5.18	0.1315	0.131	99.5
	5.20	0.1315	0.131	99.9
p-Anisidine	3.73	0.1249	0.124	99.0
	4.14	0.1382	0.138	99.8
	5.45	0.1806	0.181	100
p-Toluidine	3.63	0.1049	0.105	99.8
	3.84	0.1106	0.111	100
	5.25	0.1517	0.152	99.9
p-Toluidine[a]	16.48	0.5236	0.5232	99.9
p-Chloroaniline	3.10	0.1069	0.106	99.5
	3.05	0.1048	0.105	99.9
	5.75	0.1985	0.198	99.4
p-Chloroaniline[b]	3.10	0.1174	0.117	99.8
	3.78	0.1433	0.143	100
p-Chloroaniline[a]	10.70	0.4135	0.4045	97.8
	10.66	0.4080	0.4030	98.8
p-Bromoaniline	3.72	0.1718	0.172	100
	3.81	0.1757	0.176	100
	5.58	0.2579	0.258	100
p-Aminoacetophenone	3.43	0.1359	0.136	99.9
	3.40	0.1352	0.135	99.6
	5.05	0.2004	0.200	99.9
p-Aminoacetophenone[a]	12.61	0.5018	0.4992	99.5
p-Aminoacetophenone[b]	3.54	0.1483	0.140	94.5
	4.42	0.1826	0.175	95.8
p-Aminobenzoic acid	3.77	0.1379	0.139	100
	3.46	0.1378	0.139	101
	3.48	0.1386	0.140	101
p-Nitroaniline	5.75	0.2123	0.214	101
	3.02	0.1120	0.112	100
	5.01	0.2038	0.203	99.8
p-Nitroaniline[a]	13.56	0.5535	0.5485	99.1
m-Nitroaniline	3.93	0.1501	0.147	98.1
	3.95	0.1506	0.148	98.4
	5.52	0.2109	0.207	98.2
m-Nitroaniline[a]	12.16	0.5008	0.4978	99.4
m-Nitroaniline[a]	11.65	0.4856	0.4770	98.2
o-Nitroaniline	5.22	0.2115	0.211	99.8
	3.47	0.1347	0.140	102
	3.55	0.1436	0.143	100
o-Nitroaniline[b]	4.34	0.1717	0.176	102
	3.60	0.1454	0.147	101
	3.75	0.1535	0.154	100

[a] Potentiometric titration.
[b] Conductometric titration.

Table II. Differentiating Titrations of Substituted Aniline Pairs

Base	$HClO_4$ Used, Ml.	Base Taken, Gram	Base Found, Gram	Purity of Base, %
p-Toluidine	3.20	0.1022	0.100	98.2
p-Nitroaniline	6.08	0.2502	0.246	98.3
p-Toluidine	3.20	0.1026	0.100	97.9
p-Nitroaniline	5.85	0.2401	0.237	98.6
p-Toluidine	6.45	0.2022	0.202	100
p-Nitroaniline	3.65	0.1519	0.148	97.2
p-Bromoaniline	3.20	0.1647	0.164	100
p-Nitroaniline	3.90	0.1664	0.160	96.2
p-Bromoaniline	3.05	0.1558	0.156	100
p-Nitroaniline	4.55	0.1921	0.187	97.1
p-Bromoaniline	3.10	0.1578	0.158	100
p-Nitroaniline	3.85	0.1597	0.158	99.0
p-Bromoaniline[a] / *p*-Nitroaniline[a]	7.64	0.1820 0.1667		98.0
p-Bromoaniline[a] / *p*-Nitroaniline[a]	7.49	0.1667 0.1769		101
p-Toluidine	4.65	0.1506	0.148	98.0
p-Aminobenzoic acid	2.25	0.0986	0.092	92.6
p-Toluidine	4.75	0.1506	0.151	100
p-Aminobenzoic acid	2.50	0.1100	0.102	92.6
p-Toluidine	3.15	0.1027	0.100	97.1
p-Aminobenzoic acid	4.85	0.2017	0.198	98.0
p-Toluidine[a] / *p*-Aminobenzoic acid[a]	7.67	0.1626 0.1038		99.9
p-Toluidine[a] / *p*-Aminobenzoic acid[a]	9.87	0.2030 0.1394		100
p-Aminophenol	4.70	0.1550	0.152	98.0
p-Aminobenzoic acid	2.60	0.1034	0.106	102
p-Aminophenol	4.60	0.1542	0.149	97.1
p-Aminobenzoic acid	2.60	0.1043	0.106	102
p-Toluidine	4.00	0.1290	0.127	99.0
p-Aminoacetophenone	2.90	0.1196	0.116	97.1
p-Toluidine	3.25	0.1024	0.103	101
p-Aminoacetophenone	3.80	0.1561	0.153	98.0
p-Toluidine	3.45	0.1095	0.110	100
p-Aminoacetophenone	4.05	0.1658	0.163	98.0
p-Chloroaniline	5.30	0.2050	0.201	98.0
p-Bromoaniline	2.60	0.1347	0.133	99.0
p-Chloroaniline	5.75	0.2201	0.218	99.0
p-Bromoaniline	2.55	0.1309	0.130	100
p-Chloroaniline	4.35	0.1626	0.165	101
p-Bromoaniline	4.20	0.2196	0.215	98.0

[a] Conductometric titration.

EXPERIMENTAL

The following procedure was used for all high frequency titrations.

After the instrument was allowed to warm up for 1 hour, a 125-ml. sample of acetic acid was pipetted into the titration cell. Weighed samples of the anilines were added to the cell and the solution was stirred for 10 to 15 minutes. The buret was lowered into the cell, so that the tip was approximately 1 cm. below the surface of the solution. The initial reading of the recorder scale was noted. The titration was carried out by adding portions of the standard titrant and recording the reading on the recorder scale after each addition. A sudden increase in current increments signified an end point, after which seven or eight more readings were obtained. The recorder readings were plotted against milliliters of titrant added; the intersection of the extrapolation of the straight-line portions of the plot was taken as the end point of the titration.

A check indicated that the variation of grid current with an increase in volume of solution in the cell was negligible when the volume of solution exceeded 100 ml.

No temperature corrections have been taken into account, as the temperature did not vary by more than 2° C. from that at the time of perchloric acid standardization.

DISCUSSION AND RESULTS

High frequency titrations of 11 anilines were attempted (Table I). Various concentrations of one of the weakest bases, *p*-nitro-

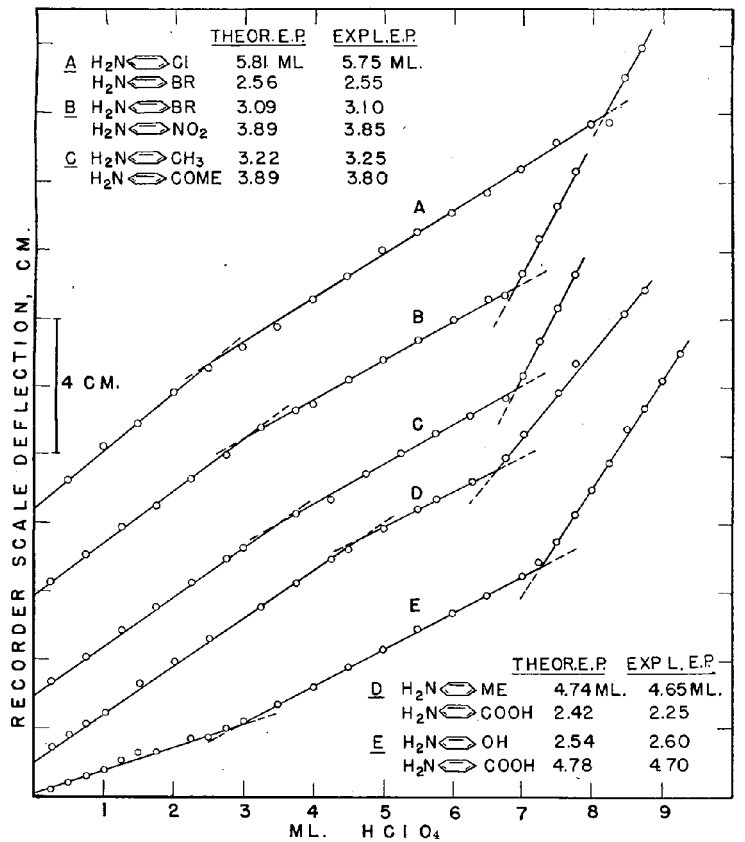

Figure 3. Differentiating titrations of mixtures of substituted anilines in glacial acetic acid

A. *p*-Cl, 1.38 × $10^{-2}M$; *p*-Br, 6.08 × $10^{-3}M$
B. *p*-Br, 7.34 × $10^{-3}M$; *p*-NO_2, 9.24 × $10^{-3}M$
C. *p*-CH_3, 7.65 × $10^{-3}M$; *p*-$COCH_3$, 9.24 × $10^{-3}M$
D. *p*-CH_3, 1.13 × $10^{-2}M$; *p*-CO_2H, 5.75 × $10^{-3}M$
E. *p*-OH, 6.04 × $10^{-3}M$; *p*-CO_2H, 1.14 × $10^{-2}M$

aniline, were titrated and the curves obtained are shown in Figure 1. Sharpness of break in the titration curve did not change when original concentrations varied from approximately 0.002 to 0.02*M* base. Figure 2 shows typical curves for six substituted anilines.

Table I also includes potentiometric and conductometric titration results for some of the same compounds. Fair agreement between the high frequency results and those obtained by the above listed methods was obtained, with the exception of *p*-aminoacetophenone. Considerable curvature was obtained in the conductometric titration for *o*-nitroaniline. Differences in sharpness of end point breaks for conductometric titration of weak bases in acetic acid media have been observed by others (*3*). The end point was not sharp in the potentiometric titration of *p*-nitroaniline. No satisfactory results were obtained by any of the three titration methods for 2,4-dinitroaniline.

Mixtures of six pairs of substituted anilines were successfully titrated for each component by the high frequency method (Table II). Figure 3 shows typical high frequency titration curves for some of these mixtures.

Titrations were unsuccessful for the following pairs: *o*- and *p*-nitroaniline, *m*- and *p*-nitroaniline, and *m*- and *p*-toluidine.

The results of titrations of pair mixtures suggest the following generalizations. Isomers of known different basicities are not resolvable—for example, *p*- and *o*-nitroanilines. Certain compounds, such as *p*-bromoaniline and *p*-toluidine, of the same basicities but varying in molecular weight are resolvable. As a rough approximation, it appears that substances differing in molecular weight by 15 units or more are resolvable, better resolution being attained if the difference in molecular weight is 30 or more. No explanation for this behavior appears tenable until more data are available. This behavior is being investigated further.

Conductometric titrations of two different pairs mixtures were made (Table II). Although excellent results were obtained for total substituted aniline content, a commercially available instrument gave no resolution for individual components in the mixture.

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RECEIVED for review April 30, 1956. Accepted July 30, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956.

Determination of the Hydrophile-Lipophile Character of Surface Active Agents and Oils by a Water Titration

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The titration by water of a dioxane-benzene solution of an oil or a surface active agent to a cloud end point can be used to determine a value related to the hydrophile-lipophile balance character of the oil or surface active agent. Details of such a titration are given and the method is compared with other rating procedures.

EMULSIONS formed with an oil and water usually require the presence of an emulsifier, if a useful stability is to be attained. Singly or in combination, hundreds of surface active agents are available which may potentially aid emulsion formation and stability. Although this variety contributes flexibility, certain principles of selection are needed, if the task of choosing an emulsifier is to be rendered manageable.

In so far as emulsifiers are concerned, theoretical considerations indicate two types of desiderata. First, there should be a proper balance, in the emulsifier, between attraction for the water phase and attraction for the oil phase. That this balance alone is not sufficient is indicated by considering the possibility of using alcohols as emulsifiers. A simple alcohol of proper balance (such as methanol or ethanol) could be found, but it would exhibit too little interfacial activity to be an effective emulsifier; it would not concentrate at the interface to a great enough degree. Secondly, one end of the emulsifier molecule should be strongly attracted by the first phase or excluded by the second and the other end of the molecule should be excluded by the first phase or attracted by the second. This ought to give rise to a high interfacial excess and very low interfacial tension. Low interfacial tension is expected to improve stability with respect to both coalescence and creaming (*7*). Improvement in creaming is anticipated because, for a given energy input in forming the emulsion, the lower the surface tension the smaller the emulsion particle size.

Of the two desiderata, only the interfacial tension has been capable of quantitative description. The other characteristic has defied such clear specification, although it has long been recognized that the balance between the polar and nonpolar portions of a surface active agent molecule is an important factor in the effectiveness of an emulsifier. Clayton (*3*) lists patents dating back to 1933 embodying this concept. The same concept is implied by some of the theories of emulsification and emulsion stability (*4–6*). Work by Schulman and Leja (*12*) on the use of solid particles in the stabilization of emulsions can also be interpreted in these terms. Winsor (*14*), in a series of papers, discusses the hydrophilic and lipophilic solvent affinities of "amphiphilic" species and the effects of these properties on hydrotropy, solubilization, and related emulsification processes. Winsor (*13*) points out the importance of the balance of these properties in spontaneous emulsification and in the establishment of the continuous phase in an emulsion.

A systematic ranking of emulsifiers and of oils with respect to the hydrophile-lipophile balance (HLB) was undertaken by Griffin (*8*). By comparing the type of emulsion (oil-water or water-oil) and the stability of emulsion formed by emulsification of a series of oils in the presence of the surface active agent, the latter was ranked. The scale chosen ranged from 1 to 40 with hydrophobic materials having a low number and hydrophilic a high number. Approximately 75 emulsions were made for the determination of each HLB number. Inspection of the table of HLB numbers indicates qualitative agreement, with expectations based on the chemical structure of a member in a given series of compounds. Thus increasing the ethylene oxide content of a polyoxyethylene ester increases the HLB number, whereas increasing the size of the hydrocarbon group decreases it.

The desirability of some measure of hydrophile-lipophile balance is then well established. Enough is known of Griffin's method to indicate that it is a time-consuming procedure. Not enough is known of this procedure to perform independent determinations of the hydrophile-lipophile balance of emulsifiers using the same scale as his, although in a recent paper Griffin (*9*) gives an equation for calculating HLB values for some types of nonionics.

The use of solubility properties offers a clue as to the hydrophile-lipophile balance of an emulsifier or oil. Solvents for use in the paint and varnish industry are ranked by tests which indicate that the solvent power of oils increases with increasing polarity and polarizability. The petroleum thinner index of 1953 (*10*) gives values of aniline point, kauri-butanol value, and composition expressed as percentage of paraffins, naphthenes, and aromatics for 400 commercial petroleum thinners. Scheflan and Jacobs (*11*) describe these tests in some detail and give tables of kauri-butanol values for pure hydrocarbons. In the kauri-butanol test, 20 grams of a solution of gum kauri in butanol is titrated with the solvent to be tested. The end point in this titration is a cloud such that 10-point Century type is illegible when viewed through a flask. One of the various standards used in this test is toluene = 105 cc. On this scale mineral spirits = 35 and solvent naphtha = 85. The aniline and mixed aniline points are the minimum critical solution temperatures of equal volumes of aniline and solvent and of two volumes of aniline mixed with one volume each of *n*-heptane and solvent, respectively (*1*).

In working with nonionic emulsifiers prepared by coupling a given alkyl phenol to a polyoxyethylene chain, it is found that longer hydrophilic chains are required for optimum emulsification in passing through the series: mineral oil, xylene, toluene, benzene. Thus an examination of the series of oils and of their preferred emulsifiers shows that the more polar the oil, the more water-soluble the preferred emulsifier (*2, 3*). Solubilities of these oils in water are of a magnitude which does not allow easy observation, however, and a magnified scale is desirable.

This report presents a study undertaken to design a solubility scale capable of ranking emulsifiers and oils in a manner commensurate with the matching that results from emulsification experiments. The ultimate purpose is to predict emulsion performance by an independent method.

EXPERIMENTAL

As the hydrophile-lipophile balance of an oil or emulsifier appeared to be related to the solubility characteristics, an index of these was sought. A titration might be used if a suitable solution of the oil or emulsifier could be titrated with a liquid of limited solubility, the end point being the appearance of a second phase.

Selection of Solvent System. In searching for a suitable solvent the requirements considered were:

1. Compatibility with a wide range of emulsifiers and, if possible, oils.
2. Ability to cover this range of materials by titration with one precipitating agent (preferably water).
3. Distinctness of the end point in terms of the spread between compounds.
4. Absence of complicating factors such as a gelation and fading of the end point.

In the preliminary work, the following procedure was arbitrarily adopted as standard.

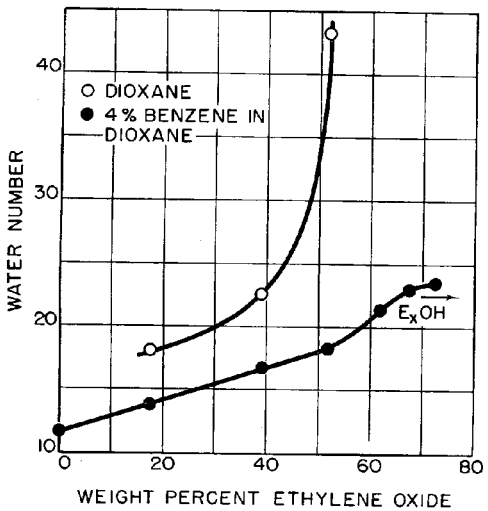

Figure 1. Octyl phenyl polyoxyethylene series

One-gram samples are accurately weighed into 125-ml. Erlenmeyer flasks, and 30 ml. of solvent is added by pipet to dissolve the sample. Distilled water is then titrated into this clear solution from a buret until the first persistent turbidity.

Using this procedure, 11 solvents were tried on various oils and emulsifiers (Table I). The uncertainties indicated in the table, and throughout the rest of this report, are mean deviations, usually obtained from but two determinations. For the octyl phenyl polyoxyethylene series the symbol OPE_x is used, the subscript referring to the number of moles of ethylene oxide appended to each alkyl phenyl radical. None of the solvents filled the requirements very well.

Because none of these compounds proved to be satisfactory as solvents, mixed solvent systems were tried. As dioxane was too hydrophilic and phenyl Cellosolve too hydrophobic when used alone, mixtures of these were investigated. The results of this investigation, given in Table II, indicated that the 50:50 solution gave too short a spread between members of the octyl phenyl polyoxyethylene series. A better range was obtained by using 25% phenyl Cellosolve in dioxane. Runs with two lots of dioxane showed that this solution gave results in satisfactory reproducibility in terms of the spacing between commercial members of the OPE_x series. At this stage both commercial dioxane and the 25% Cellosolve solution were used in other investigations.

After the 25% phenyl Cellosolve had been used for a while, it was found that the reproducibility and the distinctness of the end point were poor, particularly when ionic emulsifiers were tested. The data obtained were often unsatisfactory, and the operator was uncertain of the results and found the titrations difficult and time-consuming. In view of this, the toluene- and benzene-containing samples of dioxane were tried, as indicated in Table II. The 3% benzene solution gave some of the same trouble and the 5% benzene solution had too short a range between numbers. Optimum behavior was obtained with a 4% benzene solution, so this was accepted as the standard.

A question might be raised as to the significance of a water

Table I. Water Titration Values in Candidate Solvents

(Ml.)

Solvent	Xylene	Atreol 9	OPE_5	$OPE_{7.5}$	$OPE_{9.7}$	$OPE_{12.2}$
Dimethyl formamide	14.2 ± 1.1	0.7 ± 1	13.7 ± 0.3	>200	....	...
tert-Butyl alcohol	40.9 ± 0.1	4.2 ± 0.0	131.8 ± 0.3	>200	....	...
Butyl Cellosolve	26.9 ± 0.1	4.0 ± 0.1	>200	>200	....	...
Phenyl carbitol	6.3 ± 0.3	....	7.3 ± 0.5	7.6 ± 0.0	7.6 ± 0	9.1 ± 1
n-Hexyl carbitol	....	10.0 ± 0.3	....	.....	11.8 ± 0.4	...
Pure dioxane[a]	13.2 ± 0.1	1.4 ± 0	49.9 ± 0.2	>200	....	...
Com. dioxane	12.5 ± 0.1	1.2 ± 0	43.2 ± 0.2	>200	....	...
Phenyl Cellosolve	....	....	.....	.....	2.0	...
Methyl hexyl ketone	....	....	.....	.....	0.95	...
OPE_3	....	....	.....	.....	9.9	(very viscous)
n-Hexyl Cellosolve	....	....	.....	.....	4.35	...

[a] Purified by method of Fieser (6). The final distillation, over sodium, was through a column with a 4-foot packed section. Distillate was collected in a 0.5° C. range.

Table II. Water Titration Values in Mixed Solvents

(Ml.)

Solvent System	None	Xylene	Atreol 9	OPE_5	$OPE_{7.5}$	$OPE_{9.7}$	$OPE_{12.2}$	Blend A[a]	Blend B[a]
25% phenyl Cellosolve–75% dioxane (lot 19)	....	20.1	1.4	40.2	46.6	50.3	55.0	45.3 ± 0.3	13.6 ± 0.2
25% phenyl Cellosolve–75% dioxane (lot 2)	63.0 ± 0.5	19.0	1.0 ± 0.2	38.8	44.8	48.7	52.3	....	....
50% phenyl Cellosolve–50% dioxane (lot 2)	19.5 ± 0.1	10.6 ± 0	5.2 ± 0	16.4 ± 0.1	17.4 ± 0	17.5 ± 3	18.4 ± 0	....	....
10% toluene–90% dioxane (lot 2)	....	....	....	8.2 ± 0	8.5 ± 0	8.8 ± 0.1	8.9 ± 0		
10% benzene–90% dioxane	9.08	....	....	....	....	....	....	....	....
5% benzene–95% dioxane (lot 3)	17.85 ± 0.08	....	....	....	....	....	....	14.35 ± 0.06	12.5 ± 0.3
4% benzene–96% dioxane (lot 3)	21.7 ± 0.1	9.1 ± 0.2	1.5 ± 0.1	18.4 ± 0.1	21.4 ± 0	23.1 ± 0.3	23.6 ± 0.2	15.36 ± 0.03	12.90 ± 0
3% benzene–97% dioxane (lot 3)	29.0 ± 0.2	....	....	....	....	....	....	16.09 ± 0.01	12.3 ± 0.3
2.5% benzene–97.5% dioxane	35.0	....	....	....	....	....	....	....	....

[a] Blend of nonionic and anionic emulsifiers.

Table III. Change in Water Titration Values with Dioxane Sample Variation

Solvent	OPE_5	None	Xylene
Dioxane			
Lot 1	43.2 ± 0.2	.....	12.51 ± 0.07
	41.56 ± 0.06	.....	....
Lot 2	41.26 ± 0.02	.....	12.69 ± 0.02
Lot 3	41.8	.....	....
Lot 4	46.66 ± 0.06	.....	....
Lot 19	45.0	.....	....
4% benzene–96% dioxane			
Lot 3	18.40 ± 0.06	21.7 ± 0.1[a]	9.35 ± 0.08
	....	22.1 ± 0.02[a]	....
Lot 4	....	21.64 ± 0.04[a]	9.51 ± 0.05
	....	21.79 ± 0.05[a]	9.41 ± 0.02

[a] Composition of the ca. 4% benzene solution was adjusted to give the desired water number when first made. Second line for lot 3 was a determination 5 days after the first; for lot 4, 2 months after the first.

Table IV. End Point Experiments in Determinations of Water Titration Value

(Solvent. 25% phenyl Cellosolve in dioxane)

% Anionic	% Nonionic	First Cloud, Ml.	Dense Cloud, Ml.
12.5	87.5	9 ± 1	47.7 ± 0.2
25.0	75.0	7.1 ± 0.9	17.8 ± 0.1
37.5	62.5	7.8 ± 0.8	13.9 ± 0.3
50.0	50.0	7.9 ± 0.7	11.4 ± 0.7
62.5	37.5	6.40 ± 0.01	11.5 ± 0.2
75.0	25.0	8.2 ± 0.6	10.20 ± 0

number value greater than that of the solvent system alone (column 2 in Table II). This observation can be expected whenever the emulsifier is more hydrophilic than the solvent system used.

Because commercial dioxane contains impurities in fairly high concentration, it is important to consider the effect of batch to batch variation in dioxane on water number. That this is found, and indeed accounts for a variation from 41.3 to 46.7 for the water number of OPE_5 in dioxane as solvent, is seen in Table III. As indicated in Table III, after the first several solvent lots, batches of 4% benzene in dioxane were adjusted in benzene content to give water numbers, run on the solvent system alone, in the range 21.6 to 21.8. Data given on lot 4 dioxane indicate that the effect of aging a given sample of dioxane for 2 months is not great.

Related to the problem of selecting the solvent is the problem of obtaining a suitable end point. It was previously noted that the desired end point was a pronounced turbidity, not the first appearance of a cloud. The difference between these points is illustrated by the data obtained on a series of blends with 25% phenyl Cellosolve in dioxane as the solvent (Table IV). It is obvious that the dense cloud point is more reproducible and is the one which indicates the hydrophile-lipophile balance of the emulsifier blend. At the dense cloud point there is a sharp change from emulsion droplets just large enough to be visible to droplets too small to be seen individually. It is this change in particle size that seems to be responsible for the marked change in appearance. The nature of the change is unknown, but in appearance it is somewhat reminiscent of emulsion inversion.

In order to assess the possibility of doing the determinations in the open laboratory, the effect of temperature on water number was determined. Table V gives the water numbers of five representative solutes at 20° and 30° C. As the change is roughly 0.08 ml. per degree, it would seem that ±1° C. thermostating would be adequate but that it is not satisfactory to work at ordinary room temperature (22° to 30° C.). No explanation for the rather large temperature coefficient of the water number of the solvent system alone is offered.

Table V. Effect of Temperature

(Solvent. 4% benzene in dioxane)

Solute	30° C., Ml.	20° C., Ml.	Ml./° C.
OPE_5	18.77 ± 0.02	18.0 ± 0.3	0.08
$OPE_{9.7}$	23.1 ± 0.2	22.1 ± 0.2	0.10
Atreol 9	1.87 ± 0.02	1.36 ± 0.04	0.05
Xylene	9.92 ± 0.04	9.09 ± 0.06	0.08
E.C.[a]	7.60 ± 0.01	7.08 ± 0.08	0.05
None	22.6 ± 0.3	20.2 ± 0.2	0.24

[a] Emulsifiable concentrate containing 6 pounds of Toxaphene per gallon of kerosine.

Table VI. Effect of Speed of Titration

(Solvent. 4% benzene in dioxane)

Solute	Water Number 6 ml./min.	Water Number 1 ml./min.
$OPE_{9.7}$	22.81	22.89
Sorbitan monolaurate	7.41	7.56
40% anionic-60% nonionic	12.64	12.50

Table VII. Water Numbers of Emulsifiers

(Ml.)

Compound	Water Titration (100% Dioxane as Solvent)	Water No. (4% Benzene, 96% Dioxane)
OPE_0		11.6 ± 0.1
OPE_1	18.1 ± 0.1	13.8 ± 0
OPE_2	22.5 ± 0.1	16.7 ± 0.1
OPE_5	43.2 ± 0.2	18.4 ± 0.1
$OPE_{7.5}$	>200	21.4 ± 0
$OPE_{9.7}$		23.0 ± 0.3
$OPE_{12.3}$		23.6 ± 0.2
$(OPE_{10}\text{-}CH_2\text{-})_2$	>100	24.3 ± 0.1
$n\text{-}C_{18}H_{37}\text{-}C_6H_4\text{-}E_8$		13.1 ± 0.1
Branched $C_{12}H_{25}\text{-}C_6H_4\text{-}E_5$		15.0 ± 0.1
$tert\text{-}C_{18\text{-}24}H_{37\text{-}49}\ NE_5$[a]	8.5 ± 0.1	9.4 ± 0.2
$tert\text{-}C_{18\text{-}24}H_{37\text{-}49}\ NE_{15}$[a]	11.3 ± 0	12.0 ± 0
$tert\text{-}C_{18\text{-}24}H_{37\text{-}49}\ NE_{25}$[a]	12.6 ± 0.2	13.7 ± 0.3
n-Hexadecyl + n-octadecyl alcohol		8.4 ± 0.2
Polyethylene glycol (mol. wt. 300)($E_{8.4}$)		22.8 ± 0.0
Polyethylene glycol (mol. wt. 400)($E_{9.7}$)		23.1 ± 0.3
Polyethylene glycol (mol. wt. 600)($E_{13.3}$)		22.5 ± 0.0

[a] Ethylene oxide adduct of a primary amine.

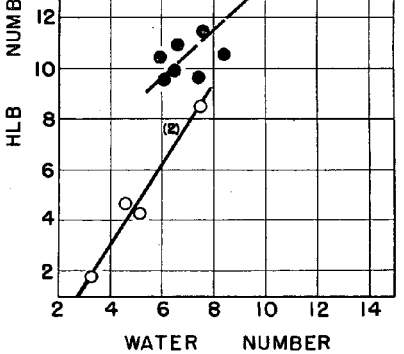

Figure 2. Polyhydric alcohol esters

The possible effect of speed of titration was investigated by taking duplicate samples and titrating one at the rate of 6 ml. per minute and the other at 1 ml. per minute. Results of this experiment (Table VI) indicate no significant effect.

Water Numbers of Surface Active Agents and Oils. The water numbers of several emulsifiers were determined as listed in Table VII. It is presumed that the low water numbers obtained for the polyethylene glycols as compared with $OPE_{12.3}$ are due to solubilization of the benzene by the latter.

The data obtained for the octyl phenyl polyoxyethylene series (OPE_x) are plotted in Figure 1, along with the titration values determined using dioxane as the solvent. It is seen (Table VII) that the effect of the presence of benzene in the dioxane is to decrease all of the titration values and to enable the method to be employed over a wider range of ethylene oxide content. Comparison of the data obtained in the two solvents for OPE_1 and the amine E_{15} indicates that the choice of solvent system will have some effect on the relative results for different families of compounds. This might be true in emulsification as well, as specific chemical factors in addition to hydrophile-lipophile balance operate in both cases. In any event, it is clear that whichever solvent is used the ordering of the members in each family is correct.

Figure 2 is a plot of HLB number (*8*) *vs.* water number for 18 surface active agents in two families of polyhydric alcohol esters: (1) ethylene oxide adducts and (2) those without ethylene oxide. A fairly good straight-line relationship between the two types of determinations is obtained for each family. The possibility exists that the HLB number for one of these families was determined by the emulsion type formed in the given oils and for the other family by emulsion stabilities. Since the HLB numbers are based on performance in emulsions, the data in Figure 2 suggest that the water number will give at least a fair indication of the performance of a surface active agent as an emulsifier.

Results of determinations of water number on a group of oils are reported in Table VIII. The table of water numbers of the oils can be interpreted simply as indicating that increasing aromatic character increases the water number. Correlation between the aniline or mixed aniline point and water number is good, except in the case of OPE_1. Thus it appears that water number parallels aniline points only when the aliphatic-aromatic balance in hydrocarbons is considered.

If surface active agents differing in hydrophile-lipophile balance range from the ones used in these tests are of interest, it is probable that a suitable spread between numbers of a series can be obtained by altering the benzene content of the solvent. Thus for determinations on OPE_x compounds with x above 10 it might be desirable to spread the upper end of the scale by going down to 3 or 3.5% benzene.

Application to Emulsifier Selection. The more polar an oil, the more hydrophilic is the best emulsifier for that oil emulsified in water. As a test of this principle and as an illustration of the utility of the water number procedure, the following experiment was performed.

A number of oil solutions of agricultural toxicants were listed in order of increasing water number (Table X).

Water numbers of blends of water-soluble emulsifier C and water-insoluble emulsifier D were determined (Table IX).

The oil solutions were emulsified in four different waters ranging in hardness from 1000 to 0 p.p.m. using the C-D emulsifier blends. A water-oil ratio of 85:15 to 96:4 by volume was used and the emulsifier content was from 3 to 6% of the weight of the oil. These ratios were determined by the field use requirements.

Emulsion stability was determined by observing the rates of creaming, clearing, and free oil formation following the procedure of Brown and Riley (*2*). From these data the best emulsifier for each oil-water system was judged. These best emulsifiers are listed in Table X.

Table VIII. Water Numbers of Oils

(Ml.)

	Water No.	Aniline[a] Pt., ° C.	Mixed Aniline[a] Pt., ° C.
Bayol N-150 (Esso)	1.01 ± 0.01	Ca. 108	..
Dormex (Cities Service)	1.3 ± 0.1	103.7	..
s/v Prorex M (Socony Vacuum)	1.43 ± 0.06	99.5	..
Atreol 9 (Atlantic)	1.49 ± 0.06	101.7	..
Insectasol (deodorized kerosine)	5.78 ± 0.09	74.8	..
Nonyl (tripropylene) benzene	6.50 ± 0.01	...	...
Velsicol AR-55 (Velsicol Corp.)	7.35 ± 0.05	...	31.5
Sovacide 544-C (Socony Vacuum)	7.57 ± 0.03	...	26.8
HAN 132 (Esso)	7.8 ± 0.1	...	25.1
Xylene	9.41 ± 0.02	...	10.8
OPE_1	13.8 ± 0	...	25.2

[a] Aniline and mixed aniline points run by H. F. Majka. Mixed aniline points run on samples whose aniline point was too low to be determined.

Table IX. Water Numbers of Blends

% Emulsifier D	% Emulsifier C	Water No.
100	0	13.2 ± 0.2
80	20	14.0 ± 0.0
70	30	14.7 ± 0.8
60	40	15.0 ± 0.1
50	50	16.1 ± 0.2
40	60	17.0 ± 0.0
0	100	23.0 ± 0.0

Table X. Performance of Blends of Emulsifier C and Emulsifier D

Toxicant–Oil Solution			% of Emulsifier C in Blend Giving Best Performance			
Lb.		Water No.	1000 p.p.m. hard water	Navy hard water	Stoneville (soft) water	Distilled water
4	Toxaphene-kerosine	6.3 ± 1	<0	..	<0	<0
6	Toxaphene-kerosine	7.1 ± 0.1	<0	<0	..	<0
6	Chlordan-kerosine	7.2 ± 0.1	<0	..	0	<0
8	Toxaphene-kerosine	7.6 ± 0.2	<0	..	0	10
	25% DDT-aromatic naphtha	7.7 ± 0.1	0=10	..	10	<0
8	Chlordan-kerosine	8.2 ± 0.2	<0	..	<0	10
	25% DDT-aromatic naphtha	8.2 ± 0.1	10	10	..	0
4	2,4-D butyl ester–aromatic naphtha	9.1 ± 0.1	40	10	..	10=20
4	2,4-D isopropyl ester–aromatic naphtha	9.3 ± 0.1	30	20	..	20
4	Toxaphene	9.3 ± 0.1	30	20	..	20
2	DDT-xylene					
	25% DDT-xylene	9.4 ± 0.2	>40	20=40	..	20
1.2	γ-BHC	9.6 ± 0.0	40	..	30	30
2	DDT-xylene					
0.8	γ-BHC	9.9 ± 0.2	50	10=20=30	..	40
1.33	DDT-xylene					
1	γ-BHC-xylene	10.0 ± 0.1	>60	..	30	40
2	2,4-D THF ester	10.2 ± 0.1	40	20=40=50	..	30=40
2	2,4,5-T THF ester-xylene					

[a] < and > indicate that in judging creaming data it appeared that less, or more, respectively, Emulsifier C would have improved the performance where further testing in the desired direction was not done.

Inspection of Table X indicates that the water number of the oil solution correlates well, although not perfectly, with the water-soluble component content of the "best" emulsifier blends. The water numbers of the toxicant-oil solutions are in the expected order in general—a saturated chlorinated hydrocarbon dissolved in kerosine is the lowest and the xylene solution of an ester the highest. Water numbers of the emulsifier blends given in Table IX are also consistent with expectations, 100% water-insoluble D being the lowest and 100% water-soluble C the highest.

Performance data in the 1000-p.p.m. water when compared with the data in the softer waters show that increasing the water hardness moves the scale for the best emulsifier towards higher water numbers. This can be explained as due to the salting-out effect which makes an emulsifier less hydrophilic in the presence of hard water than soft water.

The determination of water number offers the emulsion formulator a means of ordering oils and emulsifiers and thus of estimating how a new oil or new emulsifier fits into his past experience.

ACKNOWLEDGMENT

The authors are grateful to J. K. Beemer, P. J. Kline, and John Huey for having done much of the experimental work reported herein.

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RECEIVED for review January 13, 1955. Accepted August 2, 1956.

Refractive Index of Liquids at Elevated Temperatures

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The determination of refractive index at elevated temperatures using commercially available instruments has been hampered by the lack of reliable calibration standards. Two instruments have been constructed for the absolute determination of refractive index at elevated temperatures. One instrument is useful for determinations in the temperature range 25° to 55° C., and the other from 60° to 110° C. Data obtained using these instruments are presented for three hydrocarbons prepared by API 44 at the Carnegie Institute of Technology to serve as calibration standards and six hydrocarbons on loan from API 42 at the Pennsylvania State University. Air refractive indices of these compounds at 80° and 100° C. have been determined for six wave lengths of the visible spectrum. The accuracy with which the Eykman function, $C = (n^2 - 1)/d(n + 0.4)$, represents the relationship between refractive index and density has also been investigated for two hydrocarbon liquids from room temperature to 100° C.

THE refractive index of liquids is a physical property so easily determined with accuracy that it has become a standard for their characterization. It would appear logical, therefore, to apply it to waxes and similar materials at temperatures above their melting points. However, accurate refractive indices are difficult to obtain at temperatures much above ambient because of the difficulty of maintaining the sample and part of the measuring instrument at a uniform temperature. Every commercial refractometer exhibits its own characteristic deviations when used at elevated temperatures. Reliable calibration standards are necessary, therefore, before commercially available instruments can be used with confidence.

Hollow-prism spectrometers have been reported as best suited for the absolute determination of the refractive index of liquids (*9*, *10*). Accordingly, two such instruments were constructed. One was designed for use in the temperature range 25° to 55° C. and the other from 60° to 110° C. Both utilized a Gaertner spectrometer but were fitted with measuring prisms and constant-temperature jackets of different design. Thus the entire temperature range from 25° to 110° C. could be covered conveniently and accurately. With this equipment refractive indices were measured at elevated temperatures for three compounds of high purity supplied by API Project 44 at the Carnegie Institute of Technology and intended to serve as calibration standards. Incidental to supplying data on these standards for laboratory refractometers, refractive indices were measured at several different temperatures and wave lengths for a number of hydrocarbons which had been prepared by API Project 42 at the Pennsylvania State University (*8*). The accuracy with which the Eykman function (*1*, *2*), $C = (n^2 - 1)/d\ (n + 0.4)$ expresses the relationship between the density and refractive index of organic liquids was also investigated for two hydrocarbons.

APPARATUS AND PROCEDURE

All spectrometric methods suitable for precise refractometry involve the determination of the angular deviation with respect to some fixed direction of a collimated beam of light which has been refracted by a system of plane surfaces, usually two in number. One of the most convenient and often used methods of measurement is that of minimum deviation. Its application to the precise refractometry of liquids has been thoroughly described (*10*). In brief, the method depends upon the fact that the angles of incidence and emergence are equal when the orientation of the prism containing the liquid sample is such that the deviation of the refracted ray is a minimum. The refractive index is calculated from the angle of minimum deviation and the prism angle.

Because the former angle is determined by moving the telescope arm of the spectrometer with respect to the collimator, the method becomes inapplicable at temperatures above 50° C.,

Table I. Refractive Indices of Hydrocarbons Intended as Calibration Standards

Compound	Origin	Temp., ° C.	Wave Length, A. 6563	5893	5461	4861	4358	4047
Hexadecane	API sample 568x-5S	80	1.4078	1.4098	1.4117	1.4150	1.4191	1.4225
		100	1.3998	1.4017	1.4034	1.4069	1.4108	1.4139
trans-Decahydronaphthalene	API sample 561x-5S	80	1.4411	1.4434	1.4453	1.4492	1.4536	1.4574
		100	1.4324	1.4347	1.4367	1.4409	1.4448	1.4484
1-Methylnaphthalene	API sample 578x-5S	80	1.5805	1.5882	1.5953	1.6092	1.6274	1.5446
		100	1.5710	1.5786	1.5855	1.5990	1.6172	1.6339

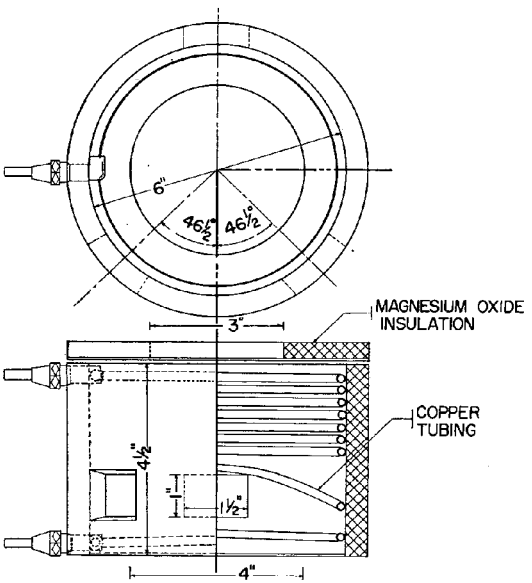

Figure 1. Air bath for refractive index measurement at moderate temperatures

for the heating jacket which is to maintain the prism and the air surrounding it at uniform temperature must have apertures large enough to allow for the maximum displacement. At high temperatures this introduces gradients of such magnitude that temperature equilibrium can no longer be maintained and a different approach is necessary. The most practical one is that described by Eykman more than half a century ago (*1*). An important characteristic of Eykman's design is the fixed angle between collimator and telescope, which makes it unnecessary to have a heating jacket with large apertures. Rotation of the prism table instead of displacement of the telescope is used to measure refractive index.

Instrument for Use Up to 55° C. A hollow prism spectrometer similar in design to that described by Tilton and Taylor (*10*) was used to determine refractive index at temperatures up to 55° C. by the method of minimum deviation. The sample was contained in a water-jacketed prism of 3-ml. capacity, which in turn was immersed in an air bath. The bath consisted of an insulated copper can, to the inside wall of which was soldered a continuous coil of copper tubing (Figure 1). Thermostated water was circulated so that it passed first through the jacket and then through the coil. Thus the sample cell was completely surrounded by air having approximately the same temperature as the cell itself. This prism and jacket were used in conjunction with a Gaertner spectrometer Model L-112. The temperature of the sample was measured using a Leeds & Northrup Type G-2 Mueller temperature bridge and platinum resistance thermometer which had been calibrated at the National Bureau of Standards.

During the minimum deviation measurements all the precautions in prism orientation and instrument alignment necessary for accurate work were carefully observed. The prism angle was measured before and after each series of double-deviation observations, each measurement involving four pointings of the telescope. Two measurements of double deviation, each involving four pointings of the telescope and utilizing different arcs of the circular scale, were included in each determination at a particular temperature. In all determinations with this apparatus a sodium arc served as source of illumination.

Instrument for Use between 60° and 100° C. For measurements in the temperature range from 60° to 100° C. an instrument based on the refractometer described by Eykman (*1*) was constructed. Only minor changes were made in the original design. Because the literature references reporting Eykman's work are not readily available, and some improvements were made, a brief description of the apparatus is given.

The essential parts of the instrument are a hollow prism containing a minimum quantity of liquid whose temperature can be precisely controlled during the measurements, and a spectrometer with a scale which permits reading angles to at least half-minutes. The latter requirement was met by the Gaertner spectrometer used in this work.

In the Eykman instrument the collimator and telescope of the spectrometer are kept fixed in position at a 40° angle with respect to each other (Figure 2). In the measurement of refractive index the prism containing the sample is turned until an image of the collimator slit falls on the center of the cross hairs in the telescope. A reading on the goniometer is taken and the prism rotated further counterclockwise until coincidence of cross hairs is observed with the Gaussian eyepiece, indicating that the telescope axis is perpendicular to the prism face. The difference in goniometer readings corresponding to these two prism positions gives the angle of emergence, J'. From this angle and the prism angle, the index of refraction of the material in the prism can be calculated by the equation:

$$n = \sin J \{1 + [(\sin J'/\sin J) + \cos \phi]^2/\sin^2 \phi\}^{1/2}$$

where $J = \phi + 40° - J'$ and ϕ = prism angle

With a constant angular deviation of 40° and a prism angle of 50° refractive indices over the range from 1.3300 to 1.6732 can be measured. The prism can also be turned clockwise and another position found where the image of the slit falls on the cross hairs of the telescope. The slit image observed in the first position forms a wider band than that observed in the second position. The more refractive the material in the prism, the less the two positions differ from the angle of minimum deviation, and the less pronounced is the difference between the band widths. Because of this difference, the refractive index calculated from the position of smaller band width should be weighed more than that calculated from the other.

The 50° prisms (Figure 3) are constructed of borosilicate glass and are supported on a glass rod. The body of the prisms consists of a hollow glass bulb in which two precision cuts for insertion of the windows were made at the proper angle. The prism faces are optically flat disks of 1-cm. diameter and 1-mm. thickness. They were mounted onto the holes ground in the bulb with either Sauereisen or Syndeticon cement. The latter is the same fish glue which Eykman himself used and is remarkably stable and inert. Both cements can be sufficiently softened to permit removal of the windows by soaking in boiling water for about 1 hour. The cell capacity is 1 ml. The glass rod supporting the prism is cemented into a brass disk which is retained in the prism holder in a reproducible position by means of three springs and a pin. The prism holder, which is attached to the central shaft of the spectrometer by setscrews, is provided with the standard three-screw spring-loaded table, so that both prism faces can be made perpendicular to the telescope axis.

The heating jacket (Figure 3) has at its center a hollow brass cylinder with two openings 40° apart, where the viewing tubes are attached. The axes of the viewing tubes coincide with those of the collimator and telescope. The prism is located within the central brass cylinder. Thus it is heated indirectly through the intervening air space. The jacket proper is a soldered copper container of the shape indicated in Figure 3. It is traversed in a horizontal plane by the viewing tubes which terminate in optically flat windows and by two smaller cylindrical wells near the bottom, into which 30-watt cartridge heaters are inserted. The tightly fitted cover is provided with an opening for attachment of a small water-cooled condenser. The entire unit is well insulated at the bottom by a 1/2-inch Bakelite plate and on the top and sides by layers of magnesium oxide plaster coated with shellac. It is supported by a bracket on the collimator arm of the spectrometer and by two legs attached to the spectrometer leveling screws.

By refluxing a liquid in the unit, a very constant and uniform temperature can be obtained in the central air space surrounding the prism. This temperature is always somewhat below the boiling point of the liquid because of some unavoidable heat loss. For this reason, the temperature of the sample is measured directly by means of a calibrated iron-constantan thermocouple whose hot junction is located in a thermal well within the prism itself. The thermocouple voltage is read with a Type K Leeds & Northrup potentiometer to ±1 mv., corresponding to about 0.02° C. The fact that the temperature is measured at a point within the sample is a decided advantage of this refractometer.

Because of changes in atmospheric pressure it is difficult to obtain specific temperatures or temperature reproducibility. This drawback is not serious, as over short ranges of temperature linear interpolation is very accurate. However, because it is necessary to take measurements at several temperatures around the desired one, it is a factor which lengthens the time required for a determination. The range of the instrument extends to well over 100° C. Only at about 130° C. was a slight warming of the prism table and angular scale noticeable, so well was the heating jacket insulated from the rest of the apparatus.

Density. In investigating the applicability of the Eykman function to the extrapolation of refractive index data the densities of two hydrocarbon liquids were measured from 25° to 100° C. Determinations were made in duplicate at each temperature by means of the 5-ml. twin-arm bicapillary pycnometer of Lipkin and others (*4*). The experimental technique was essentially that recommended by these authors. Temperatures were measured by means of a platinum resistance thermometer.

SOURCE OF MATERIALS

Refractive Index Measurements. Prepared by API Project at the Carnegie Institute of Technology to serve as calibration standards:

API 568-55 *n*-Hexadecane
API 561-55 *trans*-Decahydronaphthalene
API 578-55 1-Methylnaphthalene

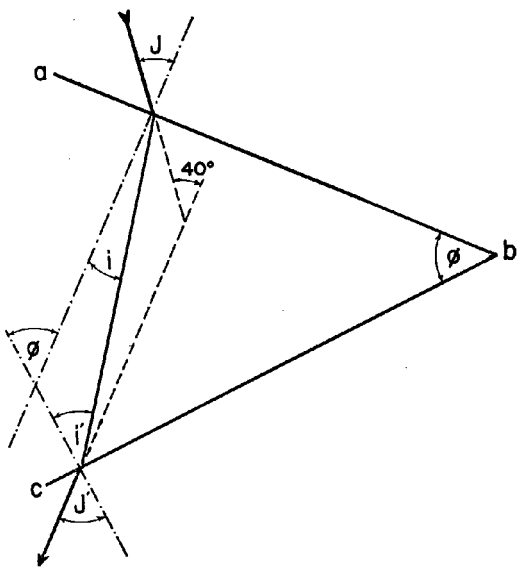

Figure 2. Geometry employed in Eykman method

J. Angle of incidence
J'. Angle of emergence
a-b. First refracting prism surface
b-c. Second refracting prism surface
i. Angle of refraction on *a-b*
i'. Angle of incidence on *b-c*
ϕ. Prism angle

Table II. Refractive Indices of Hydrocarbons from API Project 42 (*8*)

Compound	Origin	Temp., ° C.	Wave Length, A. 6563	5893	5461	4861	4358	4047
2,2,4,15,17,17-Hexamethyl-7,12-di-(3,5,5-trimethylhexyl)octadecane	PSU 182	80	1.4377	1.4401	1.4420	1.4457	1.4502	1.4538
		100	1.4261	1.4284	1.4303	1.4337	1.4380	1.4414
Hexatriacontane	PSU 190	80	1.4324	1.4347	1.4367	1.4402	1.4445	1.4480
		100	1.4254	1.4276	1.4294	1.4329	1.4371	1.4406
1-Phenyleicosane	PSU 99	80	1.4541	1.4569	1.4593	1.4640	1.4698	1.4747
		100	1.4466	1.4495	1.4519	1.4563	1.4621	1.4669
1-(1-Naphthyl)pentadecane	PSU 174	80	1.5008	1.5053	1.5029	1.5168	1.5269	1.5359
		100	1.4933	1.4976	1.5015	1.5091	1.5190	1.5278
1-Cyclohexyleicosane	PSU 100	80	1.4372	1.4394	1.4414	1.4450	1.4495	1.4531
		100	1.4300	1.4323	1.4342	1.4376	1.4420	1.4454
1-(1-Decalyl)pentadecane	PSU 175	80	1.4520	1.4544	1.4564	1.4601	1.4646	1.4686
		100	1.4449	1.4473	1.4493	1.4530	1.4574	1.4610

Table III. Data Used in Studying Reliability of Eykman Function

Temp., ° C.	Density, d_4^t	Refractive Index(n_D) by Min.Dev. Method	Eykman Constant, $(n^2-1)/d(n+0.4)$	Calcd. Refractive Index: Eykman equation using av. constant, 25–55° C.	Calcd. Refractive Index: Power[a] Series with constants based on results by Eykman refractometer, 75–105° C.
			Sample of Hexadecane		
25	0.7703	1.43262	0.74548	1.4327	1.4326
35	0.7632	1.42853	0.74570	0.4284	1.4284
45	0.7564	1.42440	0.74565	1.4243	1.4243
55	0.7496	1.42025	0.74539	1.4204	1.4201
65	0.7426	...	(Mean value)	1.4162	1.4159
85	0.7288	...	0.74556	1.4080	1.4076
95	0.7214	...		1.4036	1.4034
100	0.7176[b]	...		1.4013	1.4013
			Sample of *cis*-Decalin		
25	0.8930	1.47884	0.70749	1.4787	1.4787
35	0.8855	1.47440	0.70722	1.4745	1.4744
45	0.8777	1.47003	0.70734	1.4700	1.4700
55	0.8702	1.46572	0.70731	1.4657	1.4656
65	0.8727	...	(Mean value)	1.4615	1.4612
85	0.8472	...	0.70734	1.4526	1.4523
95	0.8391	...		1.4481	1.4479
100	0.8351[b]	...		1.4458	1.4456

[a] Power series for (impure) samples. $n = 1.44302 - 0.0004170t$ for hexadecane and $n = 1.48940 - 0.0004256t - 0.000000123t^2$ for *cis*-Decalin.
[b] Extrapolated from other density data.

Prepared by API Project 42 at Pennsylvania State University (*8*)

PSU 182 2,2,4,4,15,17,17-Hexamethyl-7,12-di(3,5,5-trimethylhexyl)octadecane
PSU 90 *n*-Hexatriacontane
PSU 99 1-Phenyleicosane
PSU 174 1-(1-Naphthyl)pentadecane
PSU 100 1-Cyclohexyleicosane
PSU 175 1-(1-Decalyl)pentadecane

Refractive Index and Density Measurements. *n*-Hexadecane, Lot 33, Humphrey Wilkinson, Inc., New Haven, Conn., was used without further purification.

cis-Decalin. Eastman Kodak practical grade Decalin was percolated through silica gel to remove any aromatic contaminants. The dearomatized Decalin was then fractionated to remove most of the lower boiling trans isomer, and 1500 ml. of the bottoms from this distillation was charged to a 25 mm. × 48 inch Podbielniak still. The cuts were examined for the presence of the trans isomer by infrared spectrometry. Those showing no contamination were combined to give about 1 liter of substantially pure *cis*-Decalin.

PRESENTATION AND DISCUSSION OF DATA

Refractive indices with respect to air at two temperatures and six wave lengths for the three hydrocarbons intended as calibration standards are given in Table I. *n*-Hexadecane, *trans*-decahydronaphthalene, and 1-methylnaphthalene were chosen as primary standards for high temperature refractometry because they are liquid at room temperature, stable, and not excessively volatile, and have refractive indices representative of the paraffinic, naphthenic, and aromatic types of hydrocarbons to which they belong.

Hydrogen, mercury, and sodium arcs were used as sources for the spectral lines. The refractive indices at 80° and 100° C. shown in Table I were calculated by linear interpolation from values experimentally determined at three or more temperatures using the Eykman instrument. As the temperature–refractive index curves were found to be straight lines for these compounds at the wave lengths employed, the indices at two temperatures are also sufficient information to calculate indices at any other temperatures within the range of observations (75° to 100° C.).

Table II gives the refractive indices at two temperatures and six wave lengths for the six hydrocarbons on loan from API Project 42. These data were also obtained using the Eykman refractometer.

It is estimated that the refractive index error in both sets of data does not exceed ±0.0002. Plots of refractive index *vs.* temperature and *vs.* wave length at fixed temperatures are smooth curves, indicating a high degree of internal consistency. Refractive indices of distilled water measured over the range 40° to 75° C. agreed with those reported by Tilton and Taylor (*10*) within ±0.0002. Measurements obtained on the same sample nearly 2 years apart agreed well within the stated experimental error, despite the fact that the apparatus had been completely disassembled and reassembled during this period.

From the data given in Tables I and II it is also possible to calculate relative refractive dispersions. Dispersion has been used in the petroleum industry for many years as an aid in the characterization of oil fractions (*5–7*). From an analytical point of view, the specific dispersion

$$\frac{n_F - n_c}{d} \times 10^4$$

where

n_F = refractive index at 4861 A.
n_c = refractive index at 6563 A.
d = density, all at the same temperature

is to be preferred to the dispersion itself. The specific dispersions of saturated hydrocarbons—e.g., paraffins and naphthenes—are nearly the same, irrespective of constitution or molecular weight. The introduction of double bonds increases this dispersion. Aromatic hydrocarbons show values, all of which are much higher than those of saturated molecules. Olefins have values intermediate between these two classes. Characterization utilizing dispersion is to a great extent dependent upon the availability of reliable values for pure hydrocarbons in the lubricating oil range. Such data are extremely limited, particularly for those compounds that are solid at or near room temperature. The data in Tables I and II serve as an indication of the possibilities of the Eykman refractometer in this regard. Many of

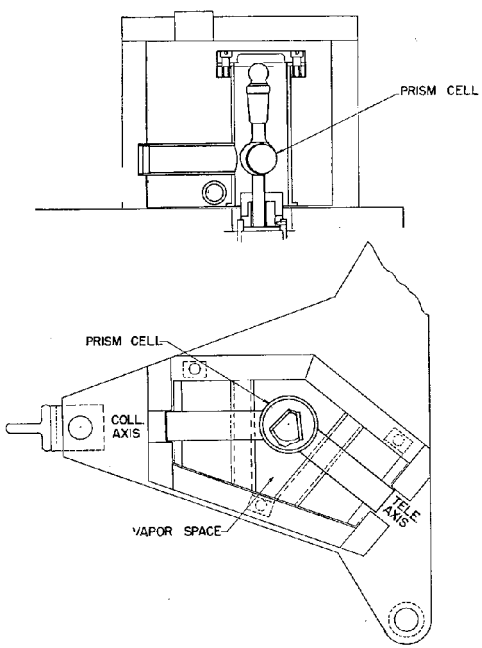

Figure 3. Prism and jacket used in Eykman method

these materials were solid at room temperature and the determination of dispersion by conventional methods was rendered virtually impossible.

The Eykman function, $C = (n^2 - 1)/d(n + 0.4)$, where C is a constant independent of temperature and pressure, has repeatedly been reported to be one of the best approximations to the actual relation between the density and refractive index of organic liquids (*2*, *3*). In order to investigate the reliability of this function, additional samples of *n*-hexadecane and *cis*-decahydronaphthalene were prepared in volumes large enough to permit the accurate determination of densities as well as refractive indices. These properties were measured over the entire range of temperature from 25° to 100° C.

In the range 25° to 55° C. refractive indices were measured by the method of minimum deviation. Temperature control was achieved using the jacketed hollow prism and air bath described. It is estimated that these data are accurate to ±0.00005, as observations on distilled water at temperatures from 25° to 55° C. showed an average deviation of ±0.00003 from reliable literature values (*10*). In the range 60° to 100° C. the Eykman instrument was used. Densities were determined in duplicate throughout the entire range and are estimated to be accurate to within two or three units in the fourth decimal.

A summary of the data obtained is given in Table III. The values of the Eykman function calculated from the data are also given. The standard deviation of both sets of values is about 0.0001. The individual values are scattered randomly about the mean. The mean values were used to calculate the refractive indices in the fifth column of the table. The average deviation between observed and calculated indices in the interval 25° to 55° C. is less than ±0.0001. In this temperature interval the best agreement would be expected, as the average value of the function was derived from the observed indices and densities. However, when the Eykman equation is used to extrapolate to temperatures in the range 65° to 100° C. the agreement between the calculated values and those obtained using the Eykman refractometer is still satisfactory, the average deviation being about two units in the fourth decimal. As further evidence of the consistency of the data it is interesting to note that the refractive index values at high temperature, when fitted by least squares methods to polynominal functions and extrapolated to lower temperatures, agreed with the values obtained by minimum deviation to within two or three units in the fourth decimal. These data indicate that the Eykman equation accurately represents the relationship between refractive index and density for the two liquids examined and that it is entirely satisfactory for converting refractive indices from one temperature to another when accurate densities are available at both temperatures.

Although neither of the instruments used is suitable for routine measurements, it is hoped that this description of their construction and operation will encourage further work of a fundamental nature in the field of high temperature refractometry.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of L. G. Bostwick, M. E. Peterkin, and Richard Williams in obtaining many of the experimental data.

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RECEIVED for review June 7, 1956. Accepted August 10, 1956.

A Thermistor Temperature Recorder

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The use of thermistors for temperature measurement and recording has been described in the literature. The present instrument is an adaptation of thermistors to the automatic continuous recording of temperatures in the range −80° to 32° C. with an accuracy within 0.05°. The span is divided into 11 ranges of 12° each, with 2° overlap on adjacent ranges. Recording of temperature is linear, making use of the fact that the temperature-resistance variation of a thermistor over relatively small intervals (12°) can be fitted to the same equation that describes the relationship between the resistance of one arm of a Wheatstone bridge and the unbalance bridge voltage. On each range of the instrument the thermistor is part of a Wheatstone bridge circuit, and the unbalance voltage is fed to a potentiometer recorder. A Leeds & Northrup recorder with a high impedance amplifier (to accommodate the 1-megohm thermistor at −80°) is used. Range selection and range changing are completely automatic.

AUTOMATIC temperature recorders are to be desired for many purposes in the fields of science and industry. Platinum resistance thermometers have been made fully automatic (*2*) and are commercially available. Recorders that employ thermocouples are similarly in use.

Although thermistors have been used for temperature measurement for a number of years, they have not been employed to any great extent as the sensing elements of recording thermometers. These temperature-sensitive resistance units possess a number of advantages which make them desirable for such work. They are produced in a wide variety of shapes and sizes, so that they are adaptable to many kinds of work, and have temperature coefficients of resistivity which are many times those of conventional resistance-thermometer materials. Depending on the type of thermistor, they may have low thermal lag, and may be of such resistance values as to make lead errors insignificant.

This paper describes an automatic temperature recorder which

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Table I. Bridge Constants for Standard Thermistor

(Ohms)

Range	R_a	R_d	Trimmer Potentiometers R_a	Trimmer Potentiometers R_d	Voltage Divider Resistor	Voltage Divider Pot.
1	2659	1,470	100	60	96.0	2
2	2256	1,900	100	100	2.7	2
3	3190	4,010	100	250	9.0	2
4	2743	5,650	100	250	2.7	2
5	2667	9,250	100	250	3.0	2
6	2599	15,100	100	900	4.4	2
7	2432	25,600	100	900	2.0	2
8	2475	47,500	100	2,500	4.0	2
9	2458	93,500	100	2,500	1.2	2
10	2369	168,000	100	10,000	...	2
11	2369	345,000	100	10,000	...	2
					(R_F) 55.0	

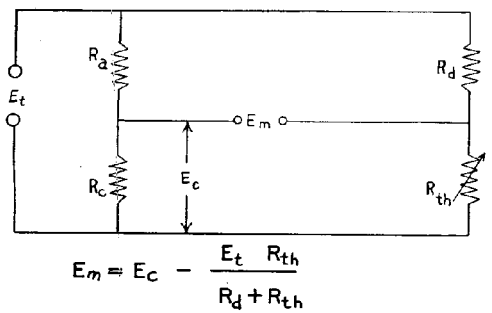

Figure 1. Wheatstone bridge circuit for thermistor

employs a thermistor as the sensing element. Most of the temperature-measuring instruments reported to date which employ thermistors are of the manual type (*3*), and require calibration curves to convert resistance readings to temperatures. A thermistor recorder has been described for use in meteorological survey in the range 46° to −12° C. with a reported accuracy within about 0.05° (*1*). The instrument reported here is an adaptation as well as an extension and refinement of that design.

DESCRIPTION OF INSTRUMENT

The completed instrument is a fully automatic temperature recorder employing a thermistor as the sensitive element. Eleven separate temperature ranges of 12° each are provided for measurement of temperatures from −80° to 32° C. Adjacent ranges overlap 2°. Temperatures are recorded on a linear scale on the chart of a Leeds & Northrup Speedomax recorder with a maximum error of 0.05°. The linear temperature scale is required to allow graphic extrapolations of time-temperature data in freezing point measurements, and graphic corrections in calorimetry.

All electrical components except the recorder unit, limit switches, and the batteries are in one chassis, the bridge unit. All adjustments can be made on the front panel or the chassis of the bridge unit, and the indicator lights are located on the front panel.

Thermistor. The thermistor selected for this work is the Western Electric Type 14B, which is recommended by the manufacturer for temperature measurement and control. The thermistor bead is enclosed in the slightly enlarged end of a glass cylinder having two tinned-wire leads brought out axially at the opposite end. The glass cylinder is approximately 2 inches long and 0.10 inch in diameter. The thermal time constant of the element when immersed in liquid is about 2 seconds. The thermistor resistance varies from about 1700 ohms at 32° C. to about 1 megohm at −80° C. The temperature coefficient is −3.9% per degree at 25° C., and is larger at lower temperatures. The resistance at the highest temperature to be measured is sufficiently high to allow the neglect of any errors due to lead resistance, while at low temperatures it is not so great that difficulties due to capacity effects and induced voltages from other electrical equipment may not be readily overcome.

It was necessary to determine very accurately the resistance-temperature characteristics of the thermistor that was to be used as the "standard" for all future calculations. The individual thermistor of the selected type (14B) was carefully chosen for reasons discussed below.

The calibration was conducted in a special cryostat assembled for this purpose. This cryostat consisted of a large and a small Dewar flask, with the smaller flask mounted inside the larger one. The inner flask was used as the measuring chamber, and contained the thermistor to be calibrated, a platinum resistance thermometer, an electric heater, and a stirrer. Both the inner flask and the space between the flasks were filled with acetone. The temperature of the acetone in the outer chamber was always held several degrees below that of the measuring chamber, to reduce heat transfer from the surroundings. The heater was used to balance small changes in temperature.

Temperature calibration points were taken at approximately 5° intervals from 32° to −80° C. Thermistor resistances were measured with a 5-decade Wheatstone bridge which had been calibrated at the National Bureau of Standards. The bridge was used in conjunction with a Leeds & Northrup 2430C galvanometer. During all calibration measurements the thermistor current was kept sufficiently small to prevent self-heating of more than 0.01° as calculated from the dissipation constant of 5 mw. per degree for the thermistor. Resistance measurements were accurate to five significant figures.

The chamber temperatures were determined with the platinum thermometer and a Mueller bridge, both of which had been calibrated. Temperatures were measured to 0.01°. The data obtained were used in calculating the resistances for the measuring circuits as described below.

CALCULATION AND SELECTION OF ELECTRICAL COMPONENTS

Thermistors are nonlinear elements, of high negative temperature coefficient, which change resistance approximately according to the equation

$$\log R = A/T + B \qquad (1)$$

where R is the electrical resistance in ohms, T is the absolute temperature in ° K., and A and B are constants. Conventional methods of using thermistors for measuring temperature require the direct determination of resistance (usually in a Wheatstone bridge circuit) and the subsequent conversion to temperature by means of a calibration curve or equation. It is, however, possible to relate the thermistor resistance to temperature by another means which is more conveniently adaptable to automation.

In a Wheatstone bridge circuit, the resistance to be measured may be directly related to the unbalance bridge voltage instead of using a null method with the bridge in balance For the case in which the resistance being measured is a thermistor, the non-

linear temperature *vs.* resistance characteristics of the element will give a bridge voltage output which is linear with temperature, if sufficiently short temperature ranges are used and correct bridge constants are chosen.

Over relatively short ranges of temperature, the temperature-resistance relationship of a thermistor may be represented by

$$t - t_1 = C - KR_{th}/(R_d + R_{th}) \qquad (2)$$

where C, K, and R_d are constants chosen to fit the experimental data exactly at three points, t_1 is the reference temperature, and R_{th} is the resistance of the thermistor at temperature t. The output (unbalance bridge voltage) of a Wheatstone bridge of the type shown in Figure 1 may be represented by

$$E_m = E_c - E_t R_{th}/(R_d + R_{th}) \qquad (3)$$

where E_m is the bridge unbalance voltage, E_c is the voltage drop across R_c (see Figure 1), and E_t is the total bridge voltage. Equations 2 and 3 are essentially the same, and if the circuit constants E_t, E_c, and R_d are chosen numerically equal to the constants in Equation 2 (K, C, and R_d), the potential drop E_m will be numerically equal to the temperature difference ($t - t_1$). This, however, would put too great a current through the thermistor. Instead, the circuit constants may be made proportional to the constants in Equation 2, and although the potential differences will not be numerically equal to the temperature differences, they will be proportional, and E_m will vary linearly with temperature. A potentiometer may be used to measure E_m, the measured voltages being equivalent to temperature readings. It can be seen from Figure 1 that E_c may be fixed by proper selection of E_t and resistances R_a and R_c. The complete method for calculating the bridge constants from experimental data is covered by Mossman, Lundholm, and Brown (*1*).

From experimental data and the method outlined above, it was found that 0.01° accuracy could be obtained over a 12° interval with a single equation. This is well within the tolerance desired for the instrument.

Figure 2 is a circuit diagram of the bridge unit including the power supply, and shows the switching arrangements and the place of the potentiometer recorder in the system. R_c is fixed at 5000 ohms for all ranges, while R_a and R_d have different values in each range. This was done so that the resistance of the bridge across which the power supply is connected will remain relatively constant, and the load on the voltage divider will not change greatly throughout the entire range of the instrument. Table I lists the values of R_a, R_d, the voltage divider resistances, and

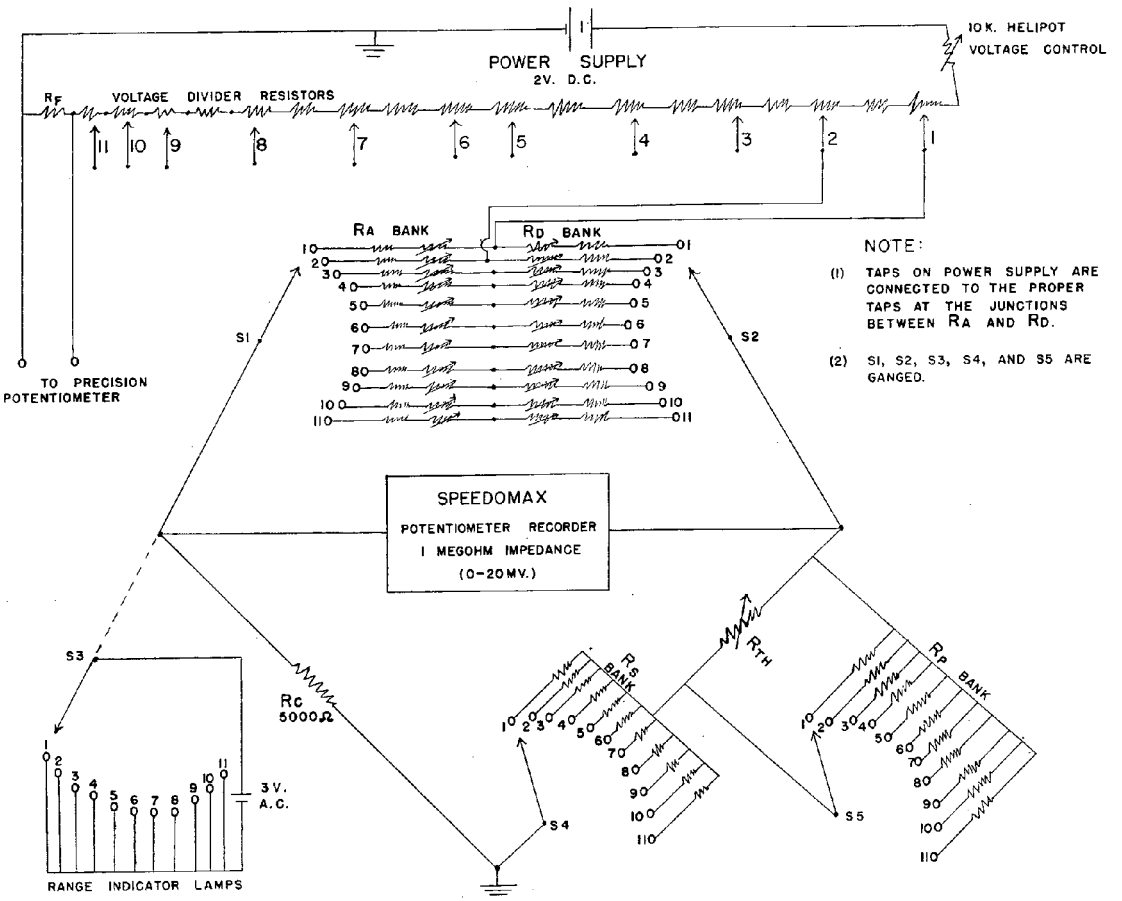

Figure 2. Circuit diagram of bridge unit

the trimmer potentiometers on each range. All bridge circuit and power supply resistors and potentiometers are wire-wound of material of low temperature coefficient.

It was found early in the development of the instrument, that dry cells were unsatisfactory to supply current either to the slide-wire of the recorder unit or to the bridge circuits, in spite of the fact that the current drain from either of these loads was 0.01 ampere or less. E_t varies from less than 0.10 volt on range 11 to about 0.18 volt on range 1. The dry cells were replaced by Willard constant-potential 2-volt storage batteries (DD 5-1), which have a capacity of 200 ampere-hours, and which were found to give very stable service for more than 6 months before recharging became necessary. Stability tests, using purely resistive loads in place of the thermistor, showed power supply stability within 0.02° for 2 months without changing the voltage standardization of the bridge unit. The storage batteries are bulky for portable use, and mercury dry cells are at present being tested for possible replacement purposes.

ADJUSTMENT AND CALIBRATION

Provision has been made on the bridge unit for connecting a precision potentiometer across a 55-ohm resistor (R_P in Figure 2) in series with the voltage divider power supply to the bridge, in order to allow a check on the bridge power supply at any time. The voltage standardization of the recorder slide-wire is performed automatically every 48 minutes as provided for by the manufacturer.

In Figure 2 it will be noted that there are small potentiometer resistances in series with R_a and R_d on each range, and that the voltage supplied to each bridge (E_t) is also adjustable. These are all available from the front panel of the bridge unit as screwdriver adjustments. They were installed because it was found that despite precautions in the selection of bridge components it was not possible to match calculated values accurately for each range. The small trimmer potentiometers allow ready attainment of 0.05° accuracy on each range. Those in series with R_a and R_d amount to 5% of the total resistance in those arms of the bridge, and provide the means of adjusting the slope of each range. Each range is individually calibrated against resistance values which were derived from the experimental calibration of the standard thermistor and Equation 1.

Adjustment of the bridge circuits is a somewhat laborious process, though with experience the time necessary is considerably reduced. From consideration of the characteristics of Wheatstone bridge circuits, it is obvious that a change in the bridge voltage, E_t, will have no effect on the zero point of any range, since the bridge is in balance, and E_m, which is the variable recorded on the chart, is zero. The maximum effect of changing E_t is observed at the extreme upper end of the scale, where the difference between the two bridge arms is at a maximum. Thus, an error in E_t will be observed as a continuously increasing positive or negative error, and can be corrected by an appropriate change in the voltage divider potentiometer for that range. The helipot is set at the start to make available the proper total voltage across the voltage divider power supply, and the adjustments on each range are then made from the E_t bank on the front panel. However, errors in E_t are rarely encountered by themselves, but are normally complicated by linearity errors caused by misadjustment of R_a and R_d, in which case the process of correction is more involved.

Errors caused by R_a and R_d can be complex, and may result in errors at any part of the scale. Any change in R_a or R_d will cause a shift in the zero end of the scale, and therefore both are adjusted to maintain the zero under all conditions. In practice a primary scan of the range is made at 2° intervals using a 0.1% precision decade resistance box instead of the thermistor. The result is considered and corrections are then made. The procedure is briefly described below, but actual experience with the instrument is required before the process is easily accomplished.

If the error is a linear one, either positive or negative, increasing toward the upper end of the scale, and the zero point of the scale is correct, an adjustment of E_t in the proper direction should correct the error. If the zero is not set, either R_a or R_d is adjusted to set this point for a preliminary run, and E_t is adjusted for correct full scale reading. After this, if an error is found in the lower part of the range, R_a is adjusted by an amount equal to the error, and in the opposite direction; R_d is changed to maintain the zero setting; and E_t is changed to obtain correct full scale reading. On the other hand, if the error is in the upper part of the scale, R_d is adjusted in the opposite direction to the error in the same manner as was R_a. The zero point is reset with R_a in this case, and E_t is adjusted for full scale reading.

This procedure will correct all misadjustments in calibration that have been encountered. Usually one or two series of approximations as described above are required for a calibration within 0.02°.

RECORDER UNIT

The recorder unit is a Leeds & Northrup Speedomax with a full scale reading of 20 mv. divided into 120 equal parts on the chart, thus reading directly in 0.1°. This recorder was ordered with an amplifier which has an input impedance of 1 megohm. This was required to prevent excessive loading of the bridge circuits and a resulting sluggishness of operation and error in measurement. This particular recorder was chosen because it was the most sensitive instrument available at the time, which would not require currents through the thermistor that would cause excessive heating. Newer recorders on the market today have higher sensitivity, and would require even less than the 0.01° self-heating of the thermistor experienced with the present setup.

A number of difficulties were encountered because of the high input impedance of the amplifier. This amplifier is sensitive to very small amounts of alternating current, and it was found necessary, wherever possible, to shield the bridge components and all exposed leads, and to connect them to a common ground in order to provide satisfactory operation. The thermistor itself is connected to a two-conductor shielded cable about 6 feet long, and the shield is grounded. Complete shielding of the thermistor cable becomes necessary at low temperatures where the resistance of the thermistor becomes large and alternating current pickup and capacity effects are increased.

RANGE SELECTION AND INDICATION

A number of methods were tried to make the range selection fully automatic; only the final solution to the problem is discussed here.

The ranges are changed by means of a five-circuit, 11-position stepping switch (Imtra Corp., Cambridge, Mass.) which is bidirectional in operation. The connections to this switch are shown in Figure 2. Sections $S1$, $S2$, and $S3$ change R_a, R_d, and the range indicator lights, respectively. The voltage divider taps are associated with R_a and R_d, so that no separate switch section is required for E_t. The fourth and fifth circuits of the switch are used in conjunction with substitute thermistors. The stepping switch is operated by 110 volts direct current for best results; this is obtained from the alternating current power line through a full-wave selenium rectifier.

The contact switches which govern the direction and number of ranges to be switched are located in the recorder unit. These switches are shown diagrammatically in Figure 3. The set of two-limit switches located at the low end of the scale is $L1$ and $L2$, and the set at the upper end of the scale is $H1$ and $H2$. $L1$ and $H1$ are set to operate within 0.5° of the ends of the scale, and $L2$ and $H2$ operate 0.25° from the ends. The range selector operates as follows.

As the temperature of the thermistor rises or falls, a point is reached where either $L1$ or $H1$ is closed, feeding current to the

appropriate coil of the stepping switch, which then shifts one range in the proper direction. If the temperature of the thermistor is within the limits of the new range, the recorder pen travels to the proper position on scale and continues recording where it left off on the preceding range. If, however, the temperature of the thermistor is changing very rapidly, or has been inserted into an environment where the temperature is several ranges removed from the range the instrument happens to be at the time, a different process takes place.

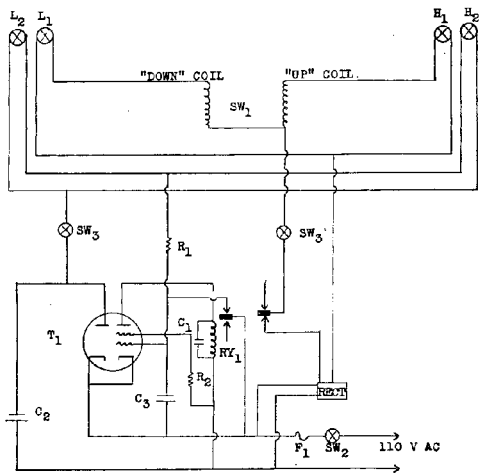

Figure 3. Range selector mechanism

C_1. 4-μf. at 450 volts
C_2, C_3. 10-μf. at 250 volts
F_1. Main fuse, 1 ampere
RECT. 500-ma. full wave selenium rectifier
R_1. 200-kilo-ohm potentiometer stepping rate control
R_2. 1000-ohm screen resistor
RY_1. 5000-ohm double-pole single-throw plate relay
SW_1. 11-position, 5-circuit, 2-way stepping switch, 110-volt d-.c. operation
SW_2. Single-pole single-throw main power switch
SW_3. Double-pole single-throw override switch
T_1. 117L/M7 GT vacuum tube

In this case, the recorder pen, instead of returning to an intermediate position on scale after closing a limit switch *L*1 or *H*1 and stepping one range in the proper direction, continues to the extreme lower or upper end of the scale, so that either *L*1 and *L*2 or *H*1 and *H*2 are both closed at the same time. When both of either set of limit switches are closed, an electronic interrupter (Figure 3) begins to operate, which alternately makes and breaks the current flowing to the stepping switch. At each interruption the switch steps one range in the proper direction. When the recorder reaches the proper range, the pen once more returns to an intermediate position, releasing both limit switches, and continues recording. The maximum speed with which the range selector can operate is dependent on the speed with which the recorder pen can return to a position where the limit switches open. This appears to be about two to three ranges per second. This process of continuous range selection normally occurs only once during a given measurement; under normal conditions only one range shift at a time is required.

ALTERNATE THERMISTORS

If any thermistor (of the same type) other than the original standard is to be used with this instrument, it is necessary not only to calibrate the new thermistor, but to calculate the values of parallel and series resistance required on each range to match the temperature-resistance characteristics of the new thermistor to those of the standard. For a new thermistor to be suitable as a substitute, it should have about the same resistance, and must have a resistance *vs.* temperature slope equal to or greater than the standard. Because of this requirement it was necessary originally to choose as the standard the thermistor of lowest $\Delta R/\Delta t$ among the several type 14B's available. There are thus 11 matching networks for each replacement thermistor. They are installed physically as a plug-in unit and electrically as shown by sections 4 and 5 of the stepping switch in Figure 2.

The method of calculating the required networks is as follows. The resistance of the unit consisting of thermistor, parallel, and series resistance is given by the standard equations

$$R_{n1} = [R_p R_{th1}/(R_p + R_{th1})] + R_s \quad (4)$$

$$R_{n2} = [R_p R_{th2}/(R_p + R_{th2})] + R_s \quad (5)$$

From these we may derive the following:

$$\Delta R_n/\Delta R_{th} = R_p^2/(R_p + R_{th1})(R_p + R_{th2}) \quad (6)$$

where R_{n1} and R_{n2} are the network resistances required at the ends of a specific range. They are equal to the resistances of the standard thermistor at the same points. R_{th1} and R_{th2} are the resistances of the replacement thermistor at the same temperatures; R_p is the parallel and R_s the series resistance required for matching. Equation 6 differs from the formula used by Mossman, Lundholm, and Brown. Their equation

$$dR_n/dR_{th} = R_p^2/(R_p + R_{th})^2 \quad (7)$$

was found unsuitable for the accuracy required in the range of temperatures involved in this work.

OPERATION

Operation of the recorder is simple, and requires little attention. All that is necessary is to connect the recorder power lead to a convenient 110-volt alternating current outlet and attach the ground wire. After the unit is turned on, a warm-up period of 5 to 10 minutes is required for the amplifier to become stabilized. As soon as this period has elapsed, the thermistor may be inserted into the cryostat, or wherever the temperature is to be measured. If it is desired to utilize the full recorder scale on any range, a switch is provided to render the range selector mechanism inoperative.

The major difference between this instrument and the one mentioned earlier (*1*) is in measuring the potential drop across the bridge circuits. Mossman, Lundholm, and Brown placed the slide-wire of the recorder in the circuit between R_a and R_c. This it can be seen that this method maintains a bridge balance by changing the ratio between R_a and R_c. This in turn continuously alters the value of E_c and the equations that express the output voltage of the bridge are no longer exact. This difficulty was avoided in the present apparatus by recording the unbalance bridge voltage directly on a recording potentiometer through the use of the high input impedance amplifier.

ACKNOWLEDGMENT

The development of the instrument took place over a period of several years. The authors wish to acknowledge the assistance of George A. Simon and Robert W. Berry, who made significant contributions to this development.

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RECEIVED for review March 17, 1956. Accepted August 3, 1956. Presented in part at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, February 27, 1956.

Modification of a Beckman Model DU Quartz Spectrophotometer for Measurements to 192 Mμ

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A Beckman Model DU quartz spectrophotometer has been modified so that the absorption spectra of solutions can be measured accurately to wave lengths as short as 192 mμ. The modification involves rotation of the collimating mirror, construction of a new wave-length scale, substitution of a photomultiplier with a fused silica window (EMI 6255) for the usual detector, replacement of the Beckman ultraviolet source with an Allen-Nester hydrogen discharge tube, use of thin-spacer absorption cells, and installation of a Vycor filter for measurement of the residual stray energy. Provision is also made for flushing the monochromator with nitrogen. Spectra obtained with the modified instrument are in excellent agreement with those measured with a recording vacuum spectrometer over the entire spectral range. The modified instrument is an adequate substitute for a vacuum spectrometer in applications of practical importance where the conventional ultraviolet spectrometer is not suitable.

FOR the past five years this laboratory has been exploring the use of far ultraviolet spectroscopy for the analysis of hydrocarbons. An earlier publication (*6*) described a recording far ultraviolet spectrometer, presented the spectra of approximately 70 pure hydrocarbons, and discussed correlations between structure and spectra. One application of these spectra has been in the determination of the types of aromatic hydrocarbons occurring in lubricating oil fractions. This method, which has been described (*4*), requires a measurement at 197.5 mμ for the estimation of substituted benzenes. As a result of this and other practical applications, it was desirable to have an inexpensive absorption spectrophotometer for routine measurements below 200 mμ.

There are many published accounts of the measurement of absorption spectra to wave lengths as short as 185 mμ with unevacuated quartz spectrometers. It seemed likely, therefore, that the widely available Beckman DU quartz spectrophotometer would be suitable for this use after only minor alterations. A brief investigation revealed that this is indeed the case and that reliable measurements with the Beckman instrument are possible to at least 192 mμ.

Extension of the useful range of the Beckman instrument involves solution of two problems. The first and most obvious of these is to alter the spectral range covered by the wave-length scanning mechanism and scale (normally 200 to 2000 mμ). The second and more serious problem is to increase the intensity and purity of the short wave-length radiation reaching the active surface of the detector.

ALTERATION OF SPECTRAL RANGE

Preliminary Mechanical Adjustment. The spectrophotometer comes equipped with a wave-length scale graduated from 200 to 2000 mμ. The calibration can be adjusted by rotation of the collimating mirror with a micrometer screw accessible through a knock-out plug in the left end of the spectrophotometer case. (The manufacturer has recently recommended resetting the prism drive arm on the prism shaft, rather than rotating the collimating mirror.) The short wave length end of the scale was made to correspond to approximately 193 mμ by rotation of this screw to its extreme position.

Table I. Calibration Data

(Wave lengths of lines from high pressure mercury arc)

True Wave Length, Mμ[a]	Observed Wave Length: Old scale after change of spectral range	Observed Wave Length: New scale (set at 546.0 mμ)	Error, mμ
194.17	201.1	194.15	−0.02
197.3	204.8	197.25	−0.05
200.2	208.3	200.2	0.0
223.0	237.5	222.9	−0.1
225.9	241.0	225.7	−0.2
230.2	246.7	230.2	0.0
232.3	249.4	232.2	−0.1
244.7	266.4	244.6	−0.1
257.6	282.7	257.6	0.0
275.3	311.2	275.4	+0.1
289.4	333.9	289.5	+0.1
296.7	346.2	296.8	+0.1
313.2	375.5	313.1	−0.1
334.2	416.5	334.1	−0.1
354.3	460.0	354.1	−0.2
366.3[b]	486.1[b]	366.2[b]	−0.1
379.0	521.5	379.0	0.0
390.6	554.0	390.5	−0.1
404.7	597.0	404.6	−0.1
435.8	705[c]	435.7	−0.1
491.6	940	491.3	−0.3
535.4	...	535.2	−0.2
546.07	1165	546.0	−0.07
579.2[b]	1283[b]	579.3[b]	+0.1
607.3		606[c]	−1.3[c]
671.6		671	−0.6
708.2		708	−0.2
737.2		737	−0.2

[a] Wave lengths from (*3*) and (*7*).
[b] Doublet averaged.
[c] Wave lengths greater than 600 mμ cannot be read with precision much better than 1 mμ.

Recalibration. A preliminary calibration was then obtained by observing the positions of easily recognized absorption bands of solutions of benzene, naphthalene, and anthracene and of vapors of 1,3-butadiene. These data were smoothed and intermediate points obtained by plotting the correction against the wave length. From this preliminary calibration curve it was possible to predict the approximate position of most of the lines in the mercury spectrum. The hydrogen discharge tube was then replaced with a high pressure mercury arc (Hanovia Alpine sun lamp No. S-311) and the exact positions of 49 mercury lines between 194.17 and 737.17 mμ were measured. These data were plotted on a large scale to permit accurate interpolation of other wave lengths.

Preparation of New Wave-Length Scale. An accurate full scale transparency of the wave-length scale of the spectrophotometer was made by conventional photographic methods. The transparency was covered with tracing paper and mounted on a transparent celluloid turntable in a light box. The new wave-length scale was laid out on the tracing paper by interpolating the correct wave lengths (as read from the large scale plot of the calibration data) between the markings of the old scale. The

markings were inked and lettered with a No. 80 Leroy template and a 00 pen.

It was expected that the new wave-length scale (except for the additional section at the short wave lengths) would correspond to a rotation of the old one through a constant angle. This was very nearly the case. However, a periodic error amounting to about 0.1 inch occurred at intervals of 180°, suggesting that the original scale might have been incorrectly centered by about this distance. The new wave-length scale was extended from 737 mμ (the mercury line to longest wave length for which accurate data were readily available) to 1000 mμ by rotating the tracing paper so that the 730-mμ marks of the new and old scale coincided except for the difference in radius. The portion of the old scale from 730 to 1000 mμ was then traced. A full scale, positive contact print of the new scale was mounted on the spectrometer scroll.

Calibration Adjustment. The calibration was completed by adjusting the collimating mirror so that the scale was correct for any one wave length. The blue (486.13 mμ) and red (656.28 mμ) hydrogen lines are probably the most convenient check points, although the mercury green line (546.07 mμ) was used in the present instance, because it was desired to measure a large number of additional mercury lines so that the accuracy of the scale might be checked more completely. The calibration data (Table I) indicate a maximum error of 0.2 mμ and an average error of 0.1 mμ or less over the entire ultraviolet region. The accuracy above 737 mμ has not been determined, but the new scale is expected to show a periodic error in this region, as discussed above.

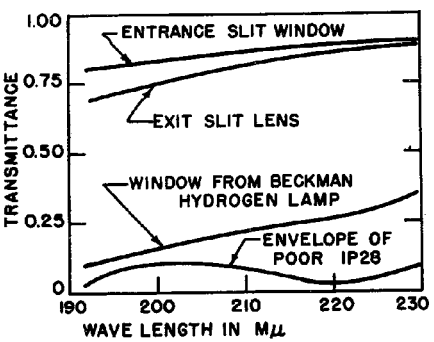

Figure 1. Absorption spectra of components of spectrometer

IMPROVEMENT OF SPECTRAL PURITY AND INTENSITY AT SHORT WAVE LENGTHS

Accurate analysis of multicomponent systems of compounds with overlapping absorption bands requires a reasonably linear relationship between absorbance and concentration of the absorbing constituents. This linear relationship can be expected only if the radiation used in the measurements is nearly "pure"—that is, comprised essentially of a single narrow band of wave lengths. The Beckman DU quartz spectrophotometer with standard ultraviolet absorption accessories meets this requirement well for measurements above 220 mμ. Below 220 mμ the radiation reaching the receiver may be very impure, so that absorption data obtained by the conventional technique may be inaccurate.

The explanation for this difficulty is simple. The hydrogen arc which serves as the source of continuous radiation in all modern ultraviolet spectrometers is rich in energy throughout the 100- to 600-mμ region. According to the best published measurements (*8*) the intensity actually increases below 210 mμ. However, before reaching the active surface of the detector the radiation must pass through the envelopes or windows of the discharge lamp, phototube, and absorption cells, as well as the sample solution or solvent and the prisms and lenses of the spectrometer. It must also undergo reflections from a number of mirrors. At each of these optical elements radiation of all wave lengths suffers some attenuation due to scattering, unwanted (or incomplete) reflections, or absorption. These losses tend to be selective, so that the shortest wave lengths are attenuated to a greater extent than the longer wave lengths.

Scattering of a small fraction of the radiation striking the prism and collimating mirror of the spectrometer results in a certain amount of undispersed radiation reaching the exit slit. In the normal operating range of a well designed instrument such as the Beckman DU spectrophotometer the spurious response due to this "spectral impurity" or "false energy" is negligible compared to the signal originating from the dispersed radiation. However, at very short wave lengths, where the dispersed radiation has suffered great attenuation, the signal from the undispersed radiation may be large compared to that from the dispersed beam. The radiation is then said to be impure or to contain a large percentage of false energy.

Measurement of False Energy. The ideal method of measuring the false energy is to determine the apparent transmittance of a material known to be completely absorbing at the wave length of interest and completely transparent at all other wave lengths. Such materials cannot be found in practice. However, for measurements below 210 mμ Vycor (a high-silica glass available from the Corning Glass Works) in a thickness of 2 mm. approximates the ideal material, as it is opaque below 210 mμ and has a high transmittance at longer wave lengths.

One of the empty openings on the filter slide of the Beckman DU spectrophotometer was fitted with a 2.05-mm.-thick, polished filter of Vycor. In the experimental work which followed, the percentage of false energy at a given spectral position below 210 mμ was assumed to be given by 100 times the ratio of the apparent per cent transmittance of the Vycor (relative to air) at the wave length in question to the apparent per cent transmittance at 280 mμ. The reference wave length, 280 mμ, was selected arbitrarily from the wide region where the transmittance of the filter is limited by reflection losses rather than by absorption. The value of the false energy computed in this manner is actually somewhat low, since the Vycor absorbs an appreciable amount of radiation above 210 mμ. A further difficulty arises in application of the false energy correction—namely, that practical samples may absorb more or less long wave length radiation than the Vycor, so that a different correction is required for each sample. As the spectral distribution of the false energy is not known, it is impossible to determine the correction with a high degree of accuracy. It is thus imperative that the correction be reduced to a minimum value.

Selection of Components. It will be obvious that reduction of the effect of stray radiation can most easily be achieved by reducing absorption of short wave-length radiation in the optical system of the spectrophotometer. Much improvement can be achieved by careful selection of accessories. The most useful criterion of performance is the false energy at 192 or 200 mμ obtained when the component in question is installed in the spectrometer.

The simple blue-sensitive phototubes originally supplied with the Beckman instrument have inadequate sensitivity for use below 220 mμ, so that a photomultiplier must be used. Examination of five 1P28 photomultiplier tubes on hand at the time this work was initiated revealed a difference of a factor of 5 in the false energy at 200 mμ measured with the best and worst of these tubes. The short wave-length response of the worst of these tubes was improved by a factor of about 2 by grinding the

envelope in front of the photocathode to about half its original thickness. This tube was later cut apart and the absorption spectrum of a part of the envelope determined (Figure 1). It will be seen that transparency is very poor below 230 mμ. Large differences in short wave-length performance were also found for different Beckman hydrogen lamps. The absorption spectrum of the window from a typical lamp is also shown in Figure 1. It is apparent that the combination of a poor photomultiplier and a poor hydrogen lamp can lead to catastrophic loss of energy below 230 mμ.

False energy may be further reduced by substitution of hydrogen lamps and photomultipliers with windows of crystal quartz or fused silica. The Allen-Nester lamp (*1*) is a commercially available hydrogen discharge tube of this type which can be installed in the Beckman instrument without much difficulty, although a special power supply must be provided. The circuit described by Allen (*1*) is satisfactory if used in conjunction with a good voltage regulator. Photomultiplier tubes with fused silica windows are available from EMI Research Laboratories, Middlesex, England, and from Dumont in this country. A tube of the former type (EMI 6255) was installed in the present instrument. The electrical circuit used by Huke, Heidel, and Fassel (*5*) with the 1P28 tube was satisfactory for this purpose.

The entrance slit window and the collimating lens which covers the exit slit of the monochromator account for loss of another 45% of the energy at 192 mμ (Figure 1). Near 200 mμ absorption by atmospheric oxygen and ozone produced near the lamp also contributes appreciably to attenuation of the monochromatic beam (Figure 2). Substantial reduction in false energy was obtained when both slit windows were removed and the monochromator was flushed with dry nitrogen. The nitrogen was introduced in the following manner: The machine screw which fastens the cast-iron monochromator case to the sheet metal base just below the collimating mirror was removed. A hole was drilled through this screw, a tubulation attached, and the screw reinserted in the base. A short length of Tygon tubing leads from the tubulation to a 1-liter Dewar flask containing fresh liquid nitrogen.

The principal remaining source of difficulty is selective short wave-length absorption by the common solvents in the usual 1-cm. absorption cells. The extent of such absorption depends, of course, on the purity of the solvent. The curves shown in Figure 3 are for secondary reference fuel grade hydrocarbons (*2*) further purified by percolation through silica gel (1 pound of gel per liter of hydrocarbon). Solvents purified in this manner have been found to be as transparent as the corresponding American Petroleum Institute spectroscopic standards. The transmittance of 1-cm. thicknesses of these solvents is, however, still poor at 200 mμ and is influenced by dissolved oxygen. The absorption by both solvent and dissolved oxygen can be reduced to a satisfactory level by reduction of the absorption cell length to less than 0.5 mm. Absorption cells of this length are not available for the Beckman instrument from commercial sources. Cells of the design used with the research vacuum spectrometer (*6*) have therefore been adopted for the present application. Fused silica—e.g., Hanovia Ultrasil—of 1-mm. thickness is satisfactory for windows in such cells, as it combines freedom from fluorescence with high transparency and good mechanical properties. Synthetic sapphire could also be used but is less suitable, since it is much more difficult to fabricate and its high refractive index leads to excessive losses by reflection. Lithium fluoride is unsatisfactory, because it fluoresces appreciably. Calcium fluoride is also very transparent in this region but has not been tested in this investigation, and in any event is much more expensive than optical grade fused silica. The absorption cells have an amalgamated lead or Teflon spacer approximately 0.1 mm. thick. The exact length of these thin cells is calculated by Beer's law from measurements of the absorbance

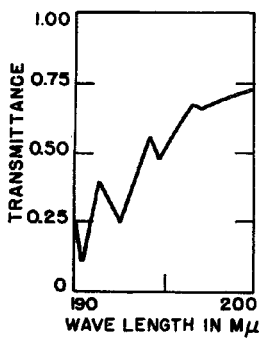

Figure 2. Absorption spectrum of 1-meter length of air at 1 atm.

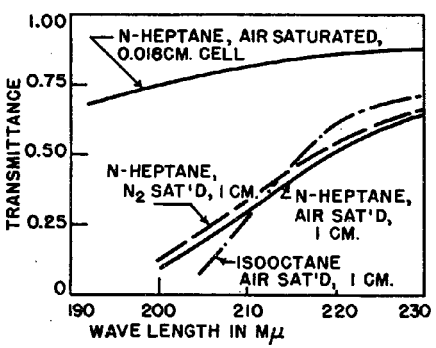

Figure 3. Absorption spectra of solvents plus absorption cells

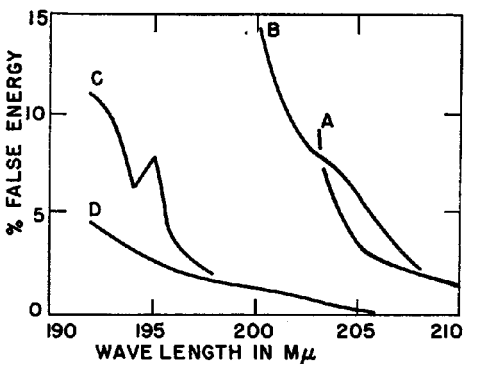

Figure 4. Effect of modifications on spectral purity

A. Spectrophotometer Serial No. D-277 with selected 1P28, selected Beckman hydrogen lamp, and 1-cm. cell filled with air-saturated *n*-heptane
B. Spectrophotometer Serial No. 1336 with same lamp, detector, and absorption cell; slit windows removed
C. Same as *B* but with 0.012-cm. cell filled with *n*-heptane
D. Spectrophotometer Serial No. 1336 with EMI 6255, Allen-Nester lamp, 0.018-cm. cell with *n*-heptane; monochromator flushed with nitrogen

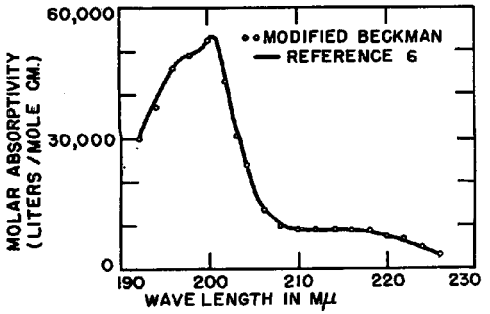

Figure 5. Absorption spectrum of 1,3-dimethyl-5-ethylbenzene

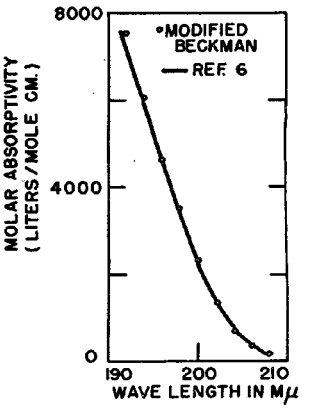

Figure 6. Absorption spectrum of *trans*-2-hexene

of a solution in the thin cell and the absorbance of a quantitatively diluted aliquot of the same solution in a standard 1.000-cm. absorption cell. It has been found practicable to match lengths of pairs of cells of this type to about 1 to 3%.

A cell compartment of conventional design was constructed of black Phenolite to accommodate two cells of this type.

The performance which was achieved with two Beckman DU spectrophotometers modified in varying degrees is shown by Figure 4.

Curve *A* was obtained with an instrument (Serial No. D-277) that had been completely overhauled about 2 years previously, and was fitted with the best Beckman hydrogen lamp and the best 1P28 photomultiplier available in the laboratory. The slit windows were still in place and standard 1-cm. cells filled with *n*-heptane were used in the measurements shown. The instrument could not be balanced at wave lengths below 203 mμ with a 2-mm. slit width. False energy was about 8% at this wave length and about 1.7% at 210 mμ. The other curves of Figure 4 were obtained with a second instrument (Serial No. 1336), which had been in use about 7 years prior to the work described here.

Curves *B* and *C* were obtained with the same selected photomultiplier and hydrogen lamp which had been used in the other instrument. The windows had been removed from the monochromator for this experiment, however. The false energies obtained with the 1-cm. cell were similar to those observed with the first instrument. With a cell 0.012 cm. in length filled with *n*-heptane, a tenfold reduction in false energy at 200 mμ was obtained and the measurements could be extended from 200 to 192 mμ, although the false energy at 192 mμ was about 11%. The maximum in false energy near 195 mμ in curve *C* corresponds to one of the Schuman-Runge absorption bands of oxygen (Figure 2). By flushing the monochromator with nitrogen and using the lamp and multiplier with silica windows it was possible to reduce the false energy at 192 mμ to about 4%. As the mirrors in this instrument were old and the Allen-Nester lamp and the EMI multiplier were randomly selected, it is possible that performance could have been improved further by selection of these components. Below 220 mμ, the mirrors rapidly lose reflectivity and can deteriorate significantly in a few months.

A practical criterion of adequate spectral purity is that calibration curves obtained with a spectrophotometer should follow Beer's law. The data of Table II show that the fully modified instrument satisfied this criterion reasonably well. The absorbance data have been corrected for the 1.4% false energy at 200 mμ as measured with the Vycor filter. It is apparent that there is a small residual nonlinearity which results in a 5% spread of absorptivities over the 0.2 to 1.0 range of absorbances. This is probably due in part to underestimation of the false energy by the Vycor filter technique and in part to nonuniformity of the thickness of the spacer over the free aperture of the absorption cells. The average molar absorptivity of 52,200 for 1,3-dimethyl-5-ethylbenzene at 200 mμ is in good agreement with the value of 54,000 ± 2700 obtained with the vacuum spectrometer (*6*).

Figure 5 shows that the spectrum of this compound measured with the modified Beckman DU is in good agreement with that from the vacuum spectrometer over the range of the former instrument. Figure 6 indicates similar agreement in the case of the spectrum of *trans*-2-hexene.

Table II. Test of Beer's Law

(1,3-Dimethyl-5-ethylbenzene[a] in 0.0183-cm. cell at 200 mμ)

Concentration, Moles/Liter × 10^4	Corrected Absorbance, $\log_{10}\frac{I_0 - 0.014\ I_0}{I - 0.014\ I_0}$	Molar Absorptivity, Liters/Mole Cm.
2.06	0.204	54,100
4.12	0.396	52,500
6.17	0.582	51,600
8.23	0.771	51,200
10.29	0.970	51,500
		Av. 52,200

[a] American Petroleum Institute standard hydrocarbon No. 566-5S. Purity, 99.89 ± 0.06 mole %.

It has been found practical to transfer quantitative methods from the vacuum spectrometer to the modified Beckman DU spectrophotometer without recalibration and without significant loss of accuracy. The modified instrument has now been in routine use for about 2 years.

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RECEIVED for review March 16, 1956. Accepted July 25, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., 1956.

Cone-Plate Viscometer

Comparison with Coaxial Cylinder Viscometer

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The cone-plate viscometer provides a rapid means of obtaining reproducible flow measurements on non-Newtonian fluids by subjecting the sample to definite uniform shear rates. The conventional coaxial cylinder viscometer suffers the disadvantages of shear rate variation across the measuring annulus, end effects, limited range of operation, and filling, cleaning, and centering difficulties. The cone-plate configuration provides constant shear conditions without introducing constructional complexity. In practice, filling, cleaning, and temperature stabilization can be completed in about 30 seconds. The flow curve recorder is capable of plotting a curve in 15 seconds with uniform shear acceleration.

THE cone-plate viscometer was designed in its original form (*4*) for starch pastes and gums used in the textile industry. The scope and accuracy have since been increased to embrace a much wider range of materials with either Newtonian or anomalous flow properties. The instrument which evolved from this development has been described in detail elsewhere (*6*).

The chief design considerations were as follows:

The operating shear rate and shear stress should be constant throughout the fluid sample.

The layer of liquid sheared in the measuring gap should be as thin as possible in order to minimize the temperature rise in the fluid at high shear rates, and also to allow rapid initial temperature stabilization.

The viscometer should be simple to set up, fill, and clean. This means that the foregoing two requirements must not introduce complexity into the construction of the measuring elements.

The rate of shear should be continuously variable over a wide range.

The viscometer should be readily adaptable for automatic flow curve recording.

The cone and plate measuring system provides a satisfactory solution to all these requirements.

Essentially, the viscometer consists of a flat plate and a rotating cone with a very obtuse angle. The apex of the cone just touches the plate surface and the fluid sample fills the narrow gap formed by the cone and plate. Both the gap width (c, Figure 1) and the linear velocity of a point, r, on the cone are proportional to the radial distance; hence, the rate of shear, which is given by the ratio of velocity, Ωr, and gap width, c, remains constant throughout, because each concentric annular element of fluid (width dr) is sheared at the same rate as the adjacent elements.

Theoretically a cone half-angle of $\psi = 6°$ gives rise to a departure of only 0.35% from shear rate uniformity. However, if ψ is greater than about 4°, errors may arise due to edge effect and, at higher shear rates, due to temperature rise within the fluid (Equation 1). In practice, an angle of only 0.3° is used, with an average gap width of about 0.05 mm., requiring a sample of about 0.1 cc. Cone angles of this order simplify the mathematical analysis of non-Newtonian flow data because the whole of the measured sample attains a uniform shear rate and, hence, a constant apparent viscosity.

Figure 2 shows the fundamental simplicity of the cone and plate measuring system compared with that of the coaxial cylinder viscometer. In the expression for viscosity, the shear stress term, $\frac{3G}{2\pi R^3}$, in dyne/cm.^{2}, and the shear rate term, $\frac{\Omega}{\psi}$, in sec.$^{-1}$, appear definitely as a ratio. In the case of the coaxial cylinder viscometer the rate of shear varies from minimum at the outer cylinder (R_o) to maximum at the inner cylinder (R_i). It is not possible to attain a constant shear rate because the cylinders must have a finite radius. In practice, if the ratio of the outer and inner cylinder radii is 1.1 to 1, the shear rate varies by 20% for a Newtonian fluid. In the case of a non-Newtonian fluid the variation could be much greater (*10*). This is about the smallest gap width which may be used with convenience, so that it is usually necessary to use an average value for the shear rate. It is, of course, necessary to eliminate the end effect due to the viscous traction on the ends of the inner cylinders by guard rings or by other means.

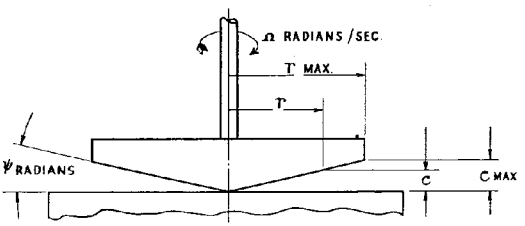


Figure 1. Method of achieving constant shear rate throughout measured sample

$$\text{Shear rate } D = \frac{\text{linear velocity}}{\text{gap width}} = \frac{\Omega r}{c}\left(\frac{\text{cm./sec.}}{\text{cm.}}\right)$$

$$D = \frac{\Omega}{\psi} \text{ sec.}^{-1}$$

These measures are not necessary in the cone and plate viscometer because the edge effect, or torque on the cone due to excess fluid round the periphery, is negligible for small cone angles. This can be shown experimentally (Figure 3) by plotting the torque on cone G against R^3 for four cones of different radius R. The cone angle, ψ, rotational velocity, Ω, and viscosity η are all constant. The data shown were obtained with a Newtonian sample fluid (silicone, $\eta = 9.1$ poises). A highly thixotropic or plastic material might conceivably give rise to a slight edge effect. However, such a substance possesses sufficient rigidity at rest to enable the excess material to be cut off flush with the cone periphery before commencing the measurement.

With a measuring gap of this kind, cooling is ensured by the large mass of the plate and cone compared with the small volume of fluid present (approximately 0.1 cc.). The dissipation of stress-induced heat can be further assisted by efficient water cooling.

The practical advantages of the cone-plate measuring system are substantial. The initial filling is rapid and temperature

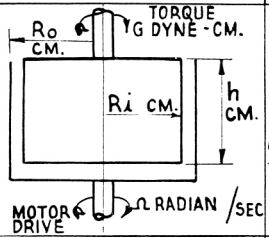
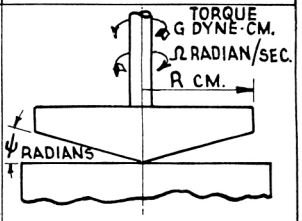
TYPE OF VISCOMETER	COAXIAL CYLINDER	CONE – PLATE
SCHEMATIC DIAGRAM		
VISCOSITY, η POISE	$\eta = \frac{G\left(\frac{1}{R_i^2} - \frac{1}{R_o^2}\right)}{4\pi h \Omega}$	$\eta = \frac{3G}{2\pi R^3} \Big/ \frac{\Omega}{\psi}$
RATE OF SHEAR, D SEC.$^{-1}$	$D_{MAX.} = \frac{2\Omega}{R_i^2\left(\frac{1}{R_i^2} - \frac{1}{R_o^2}\right)}$ $D_{MIN.} = \frac{2\Omega}{R_o^2\left(\frac{1}{R_i^2} - \frac{1}{R_o^2}\right)}$	$D = \frac{\Omega}{\psi}$
PLASTIC VISCOSITY $U = \frac{(G - G_2)S}{\Omega}$ POISE WHERE G_2 IS THE EXTRAPOLATED VALUE OF TORQUE FOR $\Omega = 0$.	$S = \frac{\left(1/R_i^2 - 1/R_o^2\right)}{4\pi h}$	$S = \frac{3\psi}{2\pi R^3}$
YIELD VALUE $f = CG_2$ DYNE/CM.^{2}	$C = S/\ln\left(\frac{R_o}{R_i}\right)$	$C = S$

Figure 2. Comparison of coaxial cylinder and cone-plate viscometers

Figure 3. Proportionality of G and R^3, indicating negligible edge effect

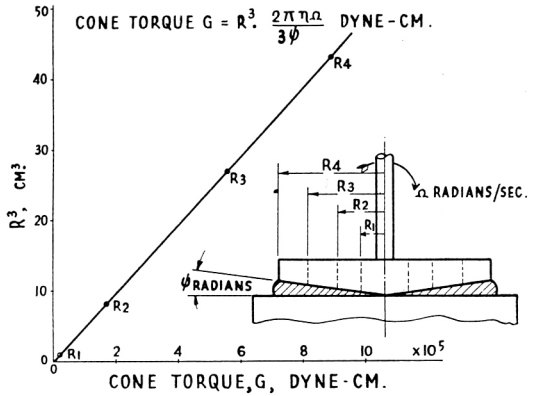
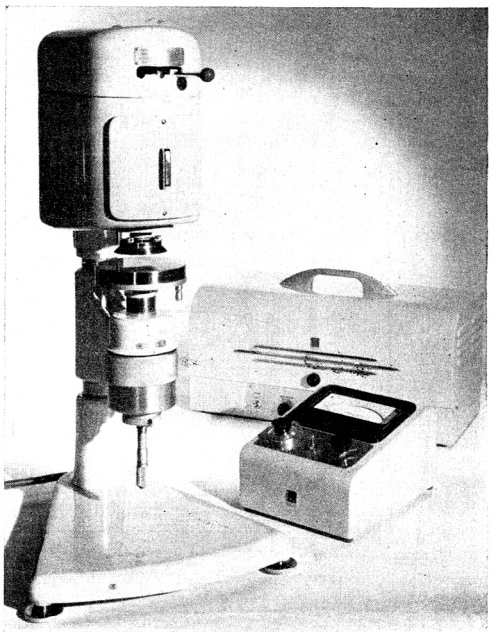


Figure 4. Cone-plate viscometer



equilibrium in the test sample is normally attained in a few seconds. With conventional rotation viscometers cleaning is generally a time-consuming operation. This present instrument, however, is cleaned by simply wiping off the flat plate and the almost flat surface of the cone.

CONSTRUCTION

The general form of the viscometer is shown in Figure 4. The measuring unit is on the left, the indicator unit in the center, and the electronic speed control amplifier for the drive motor at the right. The indicator unit houses a 10-turn potentiometer, enabling the cone speed to be set with an accuracy of better than 1%, and a five-range sensitivity switch for the measurement of cone torque. A further switch position provides precise electrical indication of contact between the cone apex and the plate in conjunction with a micrometer screw. This device facilitates the preliminary setting of the measuring gap.

Figure 5 is a simplified diagram of the measuring unit, showing the cone drive and the electro-mechanical torque measuring system (*6*). The operating height of the plate is preset by the micrometer screw, but subsequently the plate is raised and lowered by a lead screw and nut device, operated by rotating cylinder 13. It is important to have a means of reproducing automatically the upper position of the plate with a high degree of accuracy (*7*). This is achieved by spring loading a hardened flange on the plate support column against three matched steel balls. This system has been found to reproduce the plate height to within 0.0001 inch. Thermocouples in the plate surface, in direct contact with the fluid, measure the temperature rise at the boundary at high shear rates.

INFLUENCE OF STRESS-INDUCED HEAT

According to Carslaw and Jaeger (*3*) the rise in temperature, $\Delta\theta$, in an annular element in the plate surface is given by

$$\Delta\theta = \frac{r\psi H}{K}\left(\frac{\kappa t}{\pi}\right)^{1/2} \text{° C.} \qquad (1)$$

where r is the distance from the axis of rotation, t is the time in seconds, H is the heat generated per second per unit volume of liquid, and K and κ are the conductivity and diffusivity of the cone and plate, respectively.

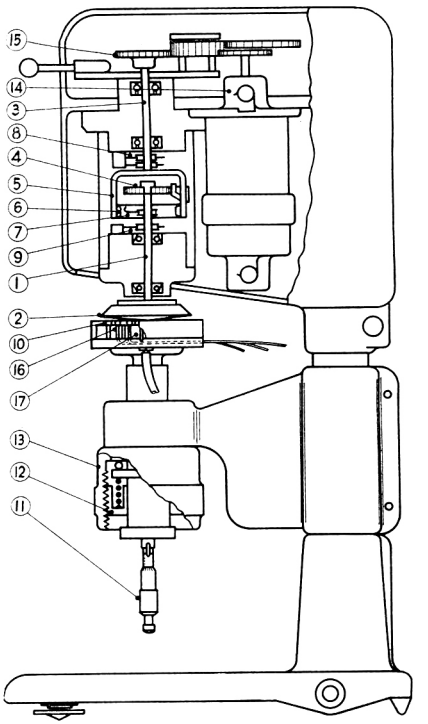


Figure 5. Schematic diagram of measuring unit

1. Cone spindle
2. Cone (included angle decreased for clarity)
3. Driving spindle
5. Bridge housing
4. Torque spring } Torque dynamometer
6. Potentiometer }
7. Wiper for potentiometer
8,9. Slip rings
10. Plate
11. Micrometer
12,13. Screw and nut, respectively, for raising plate
14. Driving motor
15. Gearing
16. Thermocouple
17. Water jacket

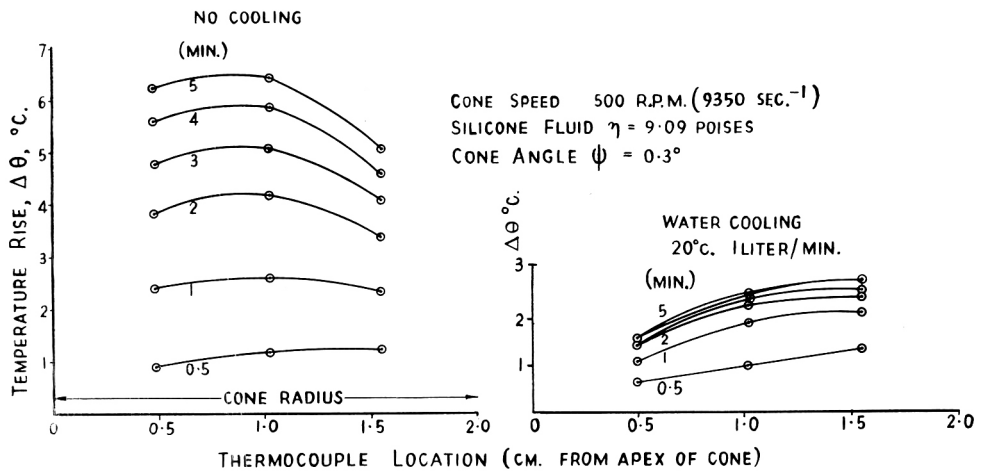


Figure 6. Radial temperature distribution at plate surface

This treatment is not rigorous, but it can be shown that the measured values of $\Delta\theta$ are in reasonable agreement with the theoretical values. Carslaw and Jaeger made the basic assumption that the cone and plate are of infinite extent so that heat is continuously conducted from the measuring gap. This does not apply in practice, but experiments with water cooling have shown that the theoretical conditions can be simulated (*6*).

The data given in Figure 6 compare the radial temperature distribution with and without water cooling, as measured by the three thermocouples in the plate surface. The time of shearing varies from 30 seconds to 5 minutes in each case. The heat developed on shearing the fluid at over 9000 sec.$^{-1}$ is sufficient in quantity and rate of generation to cause errors. The heat capacity of the plate is not large compared to the amount of heat evolved, so that, at high shear rates, water cooling is essential to prevent gradual heating of the plate. With no cooling the temperature is reduced as the cone periphery is approached. This effect is considered to be due to radial heat flow in the plate. The temperature rise is nearly proportional to the radial distance when the plate is water cooled, provided that the time of shearing is fairly short (below 1 minute).

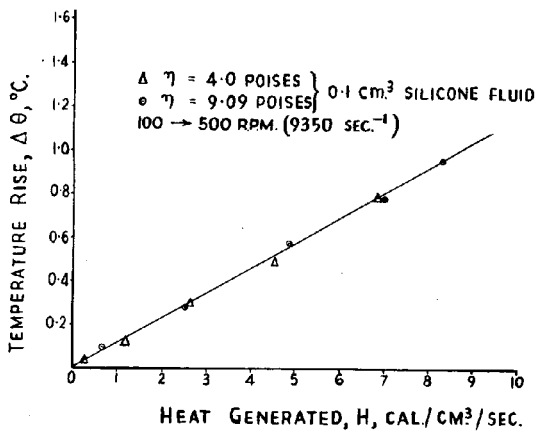


Figure 7. Measured value of $\Delta\theta$ plotted against calculated values of H

$$H = \frac{3.58 \times 10^{-5}\, G\Omega}{\pi R^3 \psi} \text{ cal./cm.}^3\text{/sec.}$$

Cone radius = 1.99 cm.; t = 1 minute; thermocouple at r = 0.5 cm.; water cooling

Figure 7 is the graph of the temperature at a point 0.5 cm. from the cone apex plotted against H, the heat generated. Torque readings were taken after shearing for 1 minute at five cone speeds. H is calculated from the torque-speed product. The experimental data are in accord with the theory. Because the volume of sample fluid is only 0.1 cc., the actual amount of heat generated is one order of magnitude below that shown on the graph. Some indication of the efficiency of the cooling system can be gained by calculating $\Delta\theta$ from the specific heat of the fluid, assuming that no heat is conducted away from the gap. This gives a temperature rise of 25° C. per second at 500 r.p.m. compared to the measured value of less than 1° C.

Up to the present no systematic work has been done to determine the temperature distribution across the fluid layer—i.e., cone to plate. Weltmann and Kuhns (*9*) have shown that, in the coaxial cylinder viscometer, the temperature rise within the fluid is greatly reduced by employing small cylinder clearances. The smallest gap which they considered was 0.5 mm. in width. This is one order of magnitude greater than the present layer thickness, so that it is reasonable to expect that no serious temperature errors are introduced by taking measurements at the boundary.

The data show that it is possible to work up to reasonably high shear rates without large temperature increases, providing that the shearing time is short. By means of an automatic recorder (*6*), a flow curve can be plotted in 15 seconds. For a top shear rate of 9350 sec.$^{-1}$, the most significant contribution to the temperature rise would occur in the last 5 seconds as maximum shear rate is approached. In this way it is possible to extend the upper limit of shear rate at which temperature compensation becomes necessary.

Flow curves can be recorded at lower cone accelerations if information is required at low rates of shear. The rate of change of cone speed is uniform and precisely reproducible, so that consistent results are given even with thixotropic materials. Flow curve recording has obvious advantages in time and labor saving. It is an arbitrary technique, normally better suited to routine process control than to fundamental studies.

If equilibrium flow curves are plotted using a point by point technique, it will sometimes be necessary to compensate for stress-induced temperature. This may be done by calculation, using the temperature coefficient of viscosity, or experimentally by cooling the sample. Lower, Walker, and Zettlemoyer (*5*) have described the latter technique using a coaxial cylinder viscometer. Both these methods are facilitated with the cone

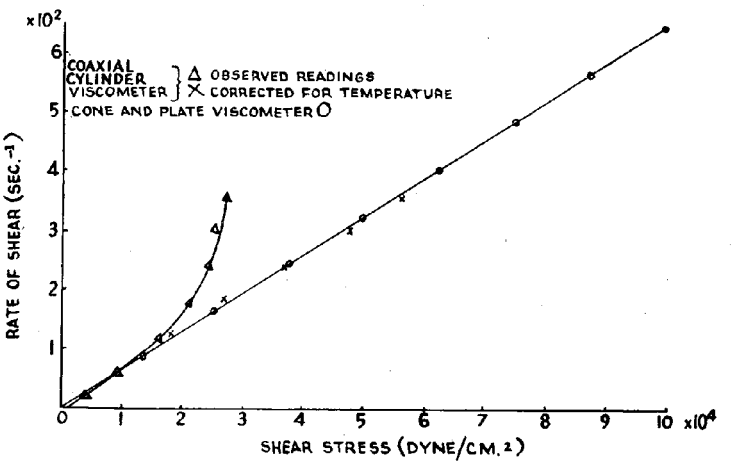


Figure 8. Influence of shear-induced temperature on performance of coaxial cylinder and cone-plate viscometers

Mineral oil, viscosity = 156 poises

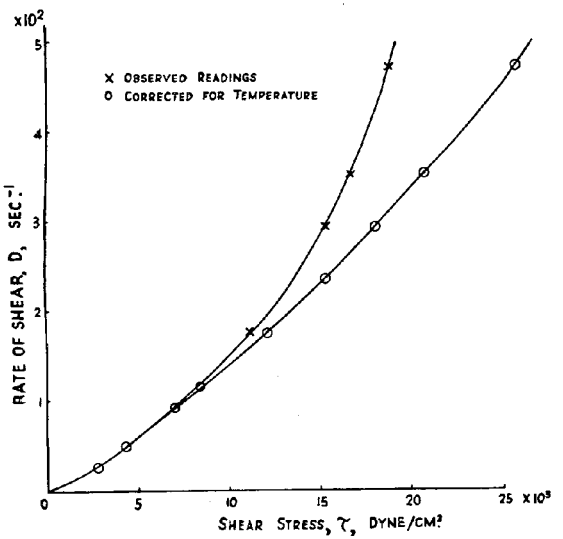


Figure 9. Coaxial cylinder viscometer

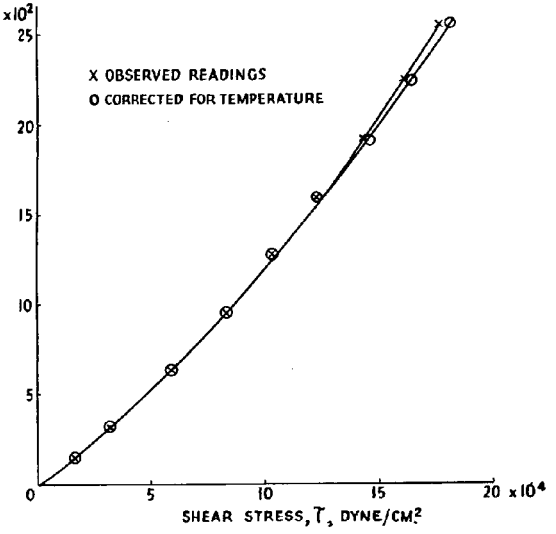


Figure 10. Cone-plate viscometer

Temperature corrections for 21% carbon black suspension in mineral oil

and plate system, since the rise in temperature is relatively lower. In many cases it has been found that no compensation is required under conditions which are known to cause considerable temperature errors in coaxial cylinder viscometers.

Figures 8, 9, and 10 compare the cone-plate instrument with a well-designed coaxial cylinder viscometer having a gap width of 0.6 mm. and a water-cooled outer cylinder. The curves of Figure 8 were obtained from a mineral oil with a viscosity of 156 poises, and a temperature coefficient of viscosity of 27% per ° C. The time between readings was 20 seconds. Appreciable temperature corrections were required by the coaxial cylinder viscometer above 100 sec.$^{-1}$ The uncorrected curve might be mistaken as an indication of non-Newtonian behavior (*8*). No noticeable temperature increase was registered in the cone-plate system at 640 sec.$^{-1}$, which was the top shear rate used.

In Figure 9 a carbon black suspension was sheared in the coaxial cylinder viscometer at increasing shear rates up to 470 sec.$^{-1}$ The temperature coefficient of viscosity was 9% per ° C. Again the temperature error became noticeable above 100 sec.$^{-1}$ Figure 10 shows a flow curve of the same material in the cone-plate viscometer. In this case the curves start to diverge at about 1600 sec.$^{-1}$, which is 16 times greater than the corresponding coaxial cylinder shear rate. The value of the cone-plate viscometer data in discriminating between shear and temperature effects is apparent.

RANGE OF OPERATION

The viscosity and shear rate requirements for an industrial viscometer may both vary over a range from 10^6 to 1. At the upper end of the range shear rates of 20,000 sec.$^{-1}$ are common in the application of paints (*1*) and printing inks, and techniques have been described (*2*) in which the graph of the logarithm of viscosity against the reciprocal square root of the shear rate is extrapolated to infinite shear rate, in order to determine the "residual" viscosity of conventional paint systems. The cone-plate viscometer can be operated up to 20,000 sec.$^{-1}$, which is high enough to justify extrapolation to infinity. With shear rates of this order it is essential to minimize the temperature rise within the fluid by reducing the volume of the sample and the size of the gap between the shearing surfaces. If necessary the temperature rise must be measured and due compensation made.

At the lower end of the shear rate range, values down to 0.01 sec.$^{-1}$ are sometimes required in order to avoid non-Newtonian flow and to determine the "zero-shear" viscosity of substances such as concentrated polymer solutions. A shear rate of 0.2 sec.$^{-1}$ can be achieved by driving the cone at 0.1 r.p.m. through reduction gearing and increasing the cone angle to 3.3°. The range can be further reduced by driving the cone with weights and measuring the angular velocity. The latter technique has been described (*7*) using a cone and plate viscometer for the investigation of the flow properties of polyisobutylene-decalin systems.

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RECEIVED for review July 14, 1955. Accepted July 9, 1956.

Rotating Concentric Tube Column

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A rotating concentric tube column has been constructed, which may be operated at either atmospheric or reduced pressures. Its performance has been evaluated under various conditions. A high capacity distillation head, an improved kettle sample thief, and a thyratron heat input controller are also described.

IN 1947, Willingham, Sedlak, Rossini, and Westhaver (*12*) described the assembly and characteristics of a rotating concentric tube distillation column which could be operated at atmospheric pressure. They indicated that such a column might be of value for the separation of many mixtures obtained in this laboratory. A more recent publication (*2*) involving a rotating column described an improved thermal rectifying column and not one operating on the principle of contact rectification. It was decided to construct a larger column than that of Willingham, Sedlak, Rossini and Westhaver, which would be able to operate at higher speeds and at reduced pressures. In addition, provision was made for automatic take-off, automatically controlled throughput, more accurate kettle sampling, and convenient operation from a central control panel.

This column was placed in operation December 1954.

CONSTRUCTION OF COLUMN

Details of the upper and lower end assemblies are shown in Figures 1 and 2, respectively. All essential dimensions are given.

The rotor was made from a stock stainless steel tubing type 304, seamless No. 2 finish, which was 4 inches in outside diameter × 0.226 inch in wall thickness. This tube was reduced to 3.962 inches in outside diameter and given a medium machined finish. End plates 0.5 inch thick were welded on, to which the steel shafts with tapered ends were attached by inserting in tapered holes and welding. This assembly was dynamically balanced and placed in an outer tube, 4.5 inches in outside diameter × 0.237 inch thick, of the same type steel. This left an annular clearance of 0.032 inch. The rotating parts weighed about 60 pounds.

The blocks which supported the lower bearing and the upper internal bearing were drilled so that liquid and vapor might pass through them. Liquid also flowed over the bearing and thus functioned as a lubricant. The upper internal bearing support block, 13, has a shoulder above the bearing and the shaft has a shoulder against the bottom of the same bearing. This eliminated end play.

Each bearing support block was threaded into the outer tube and anchored in position by Allyn setscrews, not shown in the figures. These self-aligning bearings were chosen because of their superior high-speed characteristics. The end caps were threaded on the outer tube and vacuum sealed by the application of Permatex gasket compound on the threads and by using rubber gaskets seated on steel rings which had been shrunk on the outer tube. The Permatex is hydrocarbon resistant but was gradually leeched out. Spanner wrench holes are provided in the sides of the caps. The oil-lubricated shaft seal, 6, was satisfactory and found to be

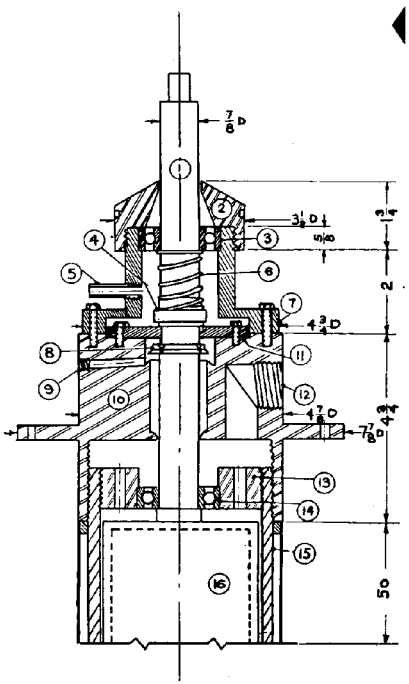

Figure 1. Rotating concentric tube distillation column, upper end

1. Shaft. Top $^{3}/_{4}$ inch is $^{1}/_{2}$ inch in diameter. Shaft is 0.9843 inch at bearings, $^{21}/_{32}$ inch between bearings; welded to rotor end plate.
2. Bearing lock nut and oil retainer
3, 14. Ball bearings, self-aligning 0.5906 inch × 0.0472 inch, 0.9843 inch in inside diameter. S.K.F. bearing No. 7205BDT
4. Rotating face of rotating shaft seal assembly, Parr No. 6357 for $^{21}/_{32}$-inch shaft
5. Oil filling tube, $^{1}/_{8}$-inch pipe, connected to oil reservoir by plastic tubing
6. Spring, rotating shaft seal
7. External bearing housing and oil chamber
8. Oil throw ring
9. Drain hole, oil chain well; threaded for $^{1}/_{4}$-inch pipe
10. Cap, upper end; threaded $1^{1}/_{2}$ inches
11. Stationary seal plate, rotating shaft seal assembly; Parr No. 6357
12. Outlet to condenser, threaded for 1-inch pipe
13. Upper bearing support block, 1.145 inches × $4^{1}/_{16}$ inches, 2.0472 inches in inside diameter
15. Outer concentric tube, stainless steel, $53^{1}/_{2}$ inches × $4^{1}/_{8}$ inches, 4.025 inches in inside diameter
16. Rotor, stainless steel, 50 inches × 3.962 inches $3^{1}/_{2}$ inches in inside diameter; $^{1}/_{2}$-inch plates welded on each end

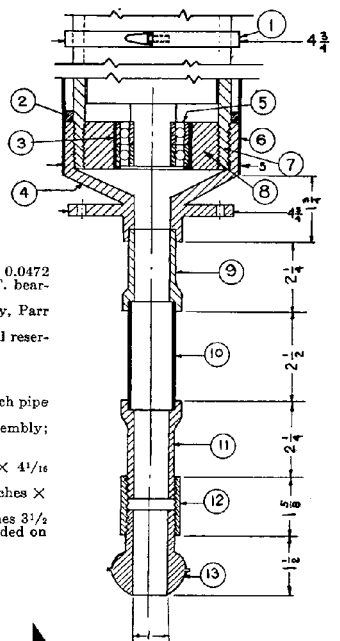

Figure 2. Rotating concentric tube distillation column, lower end

1. Brass ring, split, $^{1}/_{8}$ inch × 5 inches, $4^{1}/_{2}$ inches in inside diameter
2. Steel ring, $^{3}/_{8}$ inch × 5 inches, approx. $4^{1}/_{2}$ inches in inside diameter
3. Bearing, press fitted
4. Cap, lower end threaded $1^{1}/_{2}$ inches
5. Bearings, duplex radial thrust, 1.181 inches × 2.0472 inches, 0.9843 inch in inside diameter, S.K.F. bearing No. 1205
6. Sheet metal cylinder, $53^{1}/_{2}$ inches × 5 inches
7. Outer concentric tube
8. Lower bearing support block 1.392 inches × $4^{1}/_{16}$ inches, 2.0472 inches in inside diameter 2.0472

9, 10, 11. Flexible metal couplings

12. Sleeve, 1-inch pipe
13. Metal semiball joint, $^{60}/_{20}$

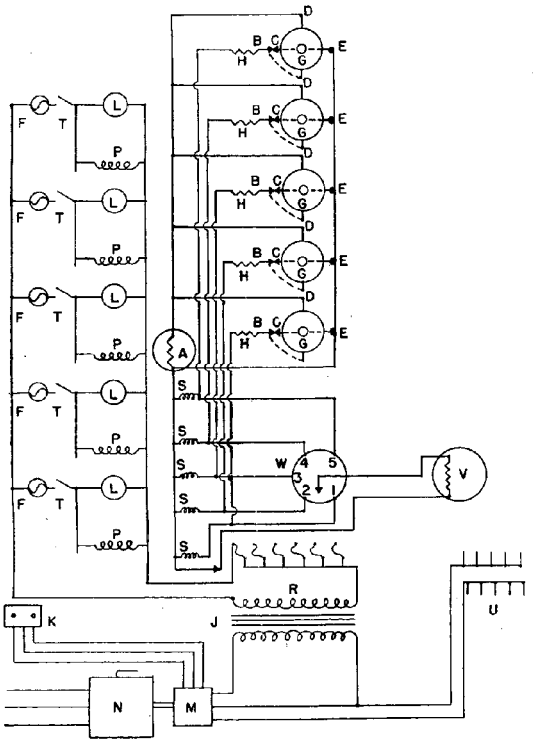

Figure 3. Power and heater circuits

A. Ammeter
B, C. Plug positions
D, E. Lines to ammeter
F. Fuses
G. Ammeter selector switches
H. Heaters
J. Constant voltage transformer
K. Start and stop buttons
L. Pilot lights
M. Magnetic switch
N. Safety switch to power supply
P. Variac, primary
R. Regulated voltage terminal block
S. Variac, secondary
T. Toggle switches
U. Unregulated voltage terminal block
V. Voltmeter
W. Voltmeter selector switch

much better than a packing gland with graphite-covered asbestos cord or a Sylphon seal which was lubricated with a mixture of cup grease and molybdenum sulfide. The seal was modified by machining the plate exactly circular and polishing its unpolished side. The gasket was placed between this latter surface and the top of the upper end cap. This seal and the external bearing were lubricated and cooled by the oil contained in the external bearing housing, 7. The external lock nut, 2, prevented the oil from splashing out. If any oil leaked past the seal, it was caught by an oil throw ring, 8, which drained into a closed receiver attached at 9. The nut was provided with spanner wrench holes. An oil reservoir was attached at tube 5. It was usually shut off to prevent the oil chamber's being emptied as a result of centrifugal force.

The external bearing housing was centered by means of tapered holes and pins, not shown, before tightening the bolts. A gasket was used between the external bearing and the top end cap. Because the external bearing was press fitted onto the shaft, jacking screws, not shown, were provided for the removal of the external bearing housing.

To prevent damage to the glass equipment attached to the column, flexible metal couplings were connected at 12 in Figure 1 and as shown in Figure 2. These flexible couplings, plus the use of metal semiball joints connecting with glass semiball joints, provided vibration-free metal-to-glass connections. A side arm leading to the head and condenser was attached at 12.

The finished column was shock mounted in a cabinet by bolting the flange of the upper cap to the top of a reinforcing plate. A $^1/_8$-inch rubber sheet was inserted between the flange and the plate and between the plate and the cabinet. The bottom of the column was held by three springs bolted to a flange which was welded to the bottom end cap. The other ends of the springs were fastened to rods bolted to a shelf within the cabinet.

COLUMN ACCESSORIES

The operation of the column was made more convenient by the centralization of the controls in a console-type panel of angle iron and Masonite. This panel was accessible through a hinged door on one end and a sliding door in the back.

Power System. A diagram of the power circuit is shown in Figure 3. The power came to the panel in a conduit containing three wires; two wires were load lines and the third a common. Between either of the load lines and the common, 115-volt potential was available. These lines were run first through a main switch inside the panel, and then through a main magnetic switch, which was operated by push buttons on the front of the panel. A load line and a common line from the magnetic switch were attached to a 2-kv. amp. Sola voltage regulator, which in turn was connected to the regulated voltage terminal block and the common terminal block. The other load line and the common line were connected to the unregulated terminal block. From these three terminal blocks came all the power for the rotating column electrical system, with the exception of the water cooler and the light inside the column mounting cabinet. An overload or power failure turned off all the power until the

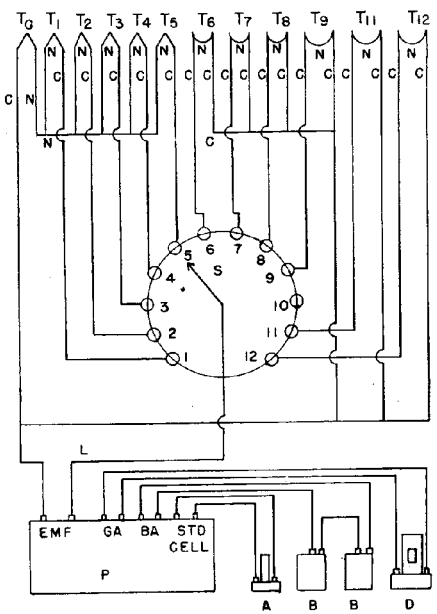

Figure 4. Thermocouple system

A. Weston standard cell, Weston Electrical Instruments Corp.
B. $1^1/_2$-volt dry cell batteries in series
C. Copper wire
D. Leeds & Northrup direct current galvanometer, No. 2284 b, Type HS; sensitivity 0.05μ volt per millimeter at 1 meter
L. Center tap line, rotary switch
N. Constantan wire
P. Leeds & Northrup K-2 potentiometer
S. Thermocouple selector switch
Tc. Thermocouple in ice-water reference cold junction
T_1 to T_{12}. Thermocouples occupying positions, as numbered on switch

magnetic switch was reset manually. The only power taken from the unregulated terminal block was that used to operate the vacuum pump.

Heater Control. The heater circuit is also shown in Figure 3. The voltage on any heater section can be shown on a voltmeter by turning the voltmeter selector switch. The amperes passing through any heater section can be read on an ammeter by plugging the jack onto the correct position at *B-C*, which simultaneously disconnects the usual connection to the common terminal block and routes the current through the ammeter. If more than one jack is plugged in, the ammeter reads the cumulative amperage.

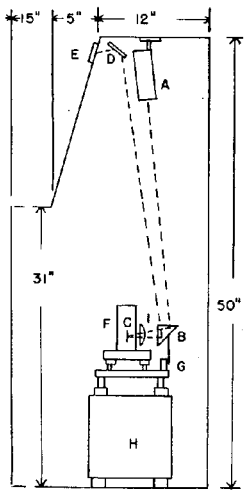

Figure 5. Optical system

A. Light source
B. Prism
C. Galvanometer mirror
D. Mirror
E. Glass scale
F. Galvanometer
G. Galvanometer and prism holder
H. 5-gallon, water-filled can
I. Focusing lens

Temperature Measurements. Eleven copper-constantan thermocouples were used at various points in the column, as indicated in Figure 4. The circuits include five thermocouples with a common ice-water cold junction and six couples which indicate temperature differentials between different parts of the column. The optical system for the galvanometer was contained within the panel and is shown in Figure 5.

Kettle Heater Regulation. After the column was brought to initial operating condition, the heat input at the kettle was regulated by use of a thyratron throughput controller. The controller regulated the current to an internal heater in the kettle according to the temperature differential between the inlet and outlet lines of the condenser coolant. Fenske, Quiggle, and Tongberg (*6*) have measured the throughput rate by using thermocouples, with the inlet line as the cold junction. They used a copper coil condenser and maintained constant water flow by means of an elevated water tank with overflow to provide a constant head. Glasebrook and Williams (*10*) recommend "suitable baffles in the condenser, to ensure the thorough mixing of the water, and proper insulation around the condenser to prevent heat loss." However, in this laboratory, no baffles or insulation were used, and the accuracy of estimating throughput proved to be better than the 5% which they reported. This accuracy was determined by the simple distillation of benzene, whose heat of vaporization is known, in such a manner that the entire condensate went into a calibrated receiver. When allowance was made for the additional cooling of the condensate as it ran down the condenser, the throughput was determined within 1 or 2% of the correct value. That insulation or vacuum jacketing of the condenser is unnecessary is shown by the fact that, when no vapor was condensing, the temperature differential usually became zero, except in extremely slow water flow rates. Tests were run with various condensers and the temperature difference was never observed to exceed 0.1° C., except when the condenser water was so cold that moisture in the air condensed on the outside. With very low values of throughput these errors become magnified, so that other methods of measuring throughput, such as counting drops from a calibrated drip tip, become more suitable.

Differential thermocouples of the condenser lines can be used to regulate a constant heat flow, which in turn controls the throughput. Thus, the throughput is constant when the molar heats of vaporization of the components being distilled are essentially equal. A commercial potentiometer controller with a sensitivity of about 4 μvolts or less could be used in connection

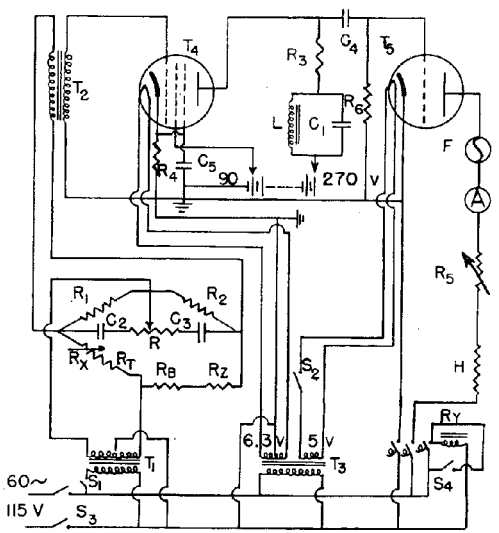

Figure 6. Thyratron temperature differential throughput control circuit

A. Ammeter, 5 amperes, alternating current
F. Fuse, 3 amperes
C_1. 0.02 mfd.
C_2. 0.5 mfd.
C_3. 0.5 mfd.
C_4. 1 mfd.
C_5. 4 mfd.
H. Kettle heater
L. Choke, 350 henrys
R_B. Thermistor, condenser inlet
R_T. Thermistor, condenser outlet
R_x. 111.1-ohm decade box
R_y. Relay
R_z. 30 ohms, 1 watt
R. 50,000-ohm potentiometer
R_1, R_2. 50 ohms
R_3. 27,000 ohms
R_4. 10,000 ohms
R_5. 50 ohms, variable
R_6. 1 megohm
S_1, S_2. Toggle switches
S_3. Power line switch
S_4. Push button switch, normally open
T_1. Thordarsen transformer, T-61 F 85; 5-volt secondary
T_2. Thordarsen transformer, T-30A 20; ratio 1 to 2
T_3. Thordarsen transformer, T-60F 94; 5-volt and 6.3-volt, center top
T_4. Vacuum tube, 6J7
T_5. Vacuum tube, 5559

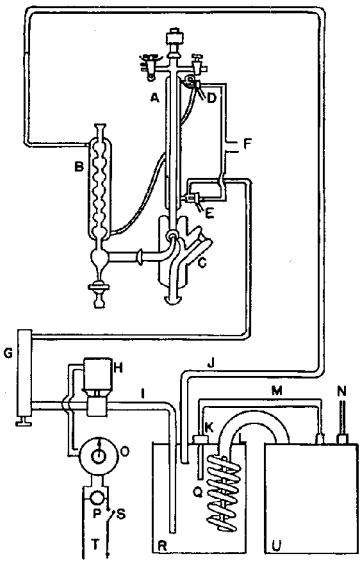

Figure 7. Water system

A. Main condenser, high speed
B. Product condenser
C. Vapor dividing distillation head
D. Upper thermistor lead wires
E. Lower thermistor lead wires
F. Thermocouple lead wires
G. Flowrotor, 0 to 1200 ml. per minute, Fischer & Porter Co.
H. Rotary vane positive pressure pump, Eastern Industries, Model VW-1
I. Inlet line, copper, $^3/_8$ inch
J. Outline line, copper, $^3/_8$ inch
K. Thermoregulator, American Instrument Co.
L. Line from refrigeration unit to cooling coils
M. Lead wires from refrigeration unit to thermoregulator
N. 110 volts, 60 cycle, unregulated
O. Variable autotransformer
P. Pilot light
Q. Cooling coil
R. Cold water tank, porcelain covered steel, insulated with styrofoam
S. Switch on panel
T. 110 volts, 60 cycle, regulated
U. Refrigeration unit, American Instrument Co.

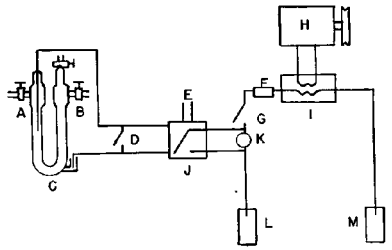

Figure 8. Motor circuit

A, B. Stopcocks
C. U-tube
D. Switch
E. Power source
F. Fuse
G. Main switch
H. Motor
I. Powerstat variable autotransformer
J. Relay
K. Pilot light
L. Regulated load terminal block
M. Common terminal block

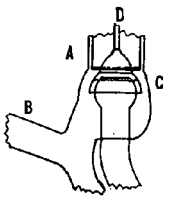

Figure 9. Distillation head

A. $^{40}/_{50}$ standard taper joint
B. Side arm
C. Take-off valve
D. Connecting rod

with the thermocouples. However, these devices are expensive, especially if proportioning rather than off-on control is desired. The circuit shown in Figure 6 was constructed in this laboratory at a cost not exceeding $35.00, and provided the desirable control.

Continuous regulation was achieved by the modification of a phase-shifting, alternating current, bridge-controlled thyratron circuit originally described by Benedict (*1*) for control of furnaces and thermostats, and later improved by Tarnopol (*9*) for control of annealing furnace temperatures.

As originally designed, the circuit employed a resistance thermometer as the temperature-sensing element, but in order to increase the sensitivity and speed of response and to lower the size and heat capacity of the sensing unit, thermistors were employed. Because thermistors have a negative temperature coefficient of resistance, the thermistor in the condenser outlet line, R_T (Figure 6), must be connected to the same arm of the bridge as the controlling decade resistance, R_x. Also, the thermistor in the inlet line, R_B, which compensates for the temperature of the incoming water, must be placed in the same position in the bridge as the resistance thermometer formerly used. To compensate for the extra resistance because of the thermistor in the decade arm, a 50-ohm resistor, R_2, was added to the resistance thermometer arm of the bridge. The thermistors used in this laboratory were obtained from the Frieze Instrument Division of Bendix Aviation Corp., Baltimore, Md., and were of the rod type with a resistance of about 57 ohms at 20° C. and a temperature coefficient of resistance of −2.0% per °C. at 20° C. The thermistor and lead wires were waterproofed with a film of silicone grease, and placed directly in the water stream in the condenser.

The thyratron will safely pass about 2.5 amperes, and because the sensitivity of the circuit is greatest when the thyratron is passing about one quarter cycle, the resistance, R_5, in the anode circuit should be set so that the maximum current which the tube will pass is about twice that required to maintain the desired throughput and in no case greater than 2.5 amperes. In practice the kettle external uncontrolled heater was set so that about 1 ampere was required to maintain the desired throughput. The rheostat, R_5, was adjusted to allow a maximum of about 2 amperes. The control point of the throughput was set by increasing the resistance of the decade, R_x, until the desired throughput was attained. To obtain maximum sensitivity the potentiometer, R, was set so as to obtain the greatest variation of anode current with variations of the decade box resistance, R_x, without the control becoming off-on. The position of the sensitivity control, R, affects the control point; therefore, it should not be changed once the control point is set by R_x. The control point was found to be quite stable. Throughputs up to 8 liters per hour were maintained for days with less than 5% variation. After shutting off the heater switches and allowing the still pot charge to become cold, the throughput rate could be quickly reestablished at the same value by turning the switches on.

The throughputs were measured by use of thermocouples also in the condenser. The feed-back lag to the control system from ordinary condenser systems was entirely too slow; therefore, two methods were evolved to increase the feed-back rate. The simplest was to reverse the water flow by running water in at the top inlet. This required the outlet line from the bottom to be held above the inlet line to keep the condenser filled. By thus having the water flow countercurrent to the vapor, the time lag while the water moves up the condenser is avoided. A superior method of increasing feed-back rate employed a condenser with high water speed. The advantage of keeping the water jacket volume small is that the time of residence in the condenser is decreased. Uniform flow of constant temperature cooling water was maintained by the use of a rotary vane-type positive pressure pump Model VW-1, from Eastern Industries Inc., New Haven, Conn. (Figure 7).

The common centrifugal-type circulating pump proved to be completely unsatisfactory. A satisfactory condition of operation involved recirculating the condenser water by pumping it through

3/8-inch copper lines from an insulated tank, in which it was cooled to a convenient temperature of 10° to 20° C. The temperature was controlled by a commercial constant temperature refrigeration device. The water flow was measured and regulated by first passing it through a rotameter with a built-in valve The voltage applied to the pump was regulated by a variable autotransformer so as not to build up too large a pressure behind the valve and overload the pump motor. A bypass valve was not used because it interfered with regulation. Water flow was maintained within 2% at rates from 300 to 1100 ml. per minute, depending on the magnitude of the throughput rate. The water system is shown in Figure 7.

Motor Assembly. The rotor was driven by a 5/8-hp. series-wound motor (Black & Decker Manufacturing Co.), capable of running up to 10,000 r.p.m. It drove the rotor with a V-belt running on a 5-inch pulley on the column shaft, and a 3 1/2-inch pulley on the motor shaft. A 1/3-hp. motor previously used was able to run the column at a 2 to 1 ratio, but was definitely overloaded. The Black & Decker motor was capable of pulling the column at speeds in excess of 6000 r.p.m. The motor circuit is shown in Figure 8. The motor speed could be continuously varied up to the maximum speed by a powerstat variable autotransformer, *I*. The power for the powerstat was taken from the regulated load terminal block, *L*, and the common terminal block, *M*. The common terminal block connected directly to the powerstat common terminal. The load line ran through a relay, *J*, then to the main switch, *G*. The fuse, *F* protected the powerstat and the motor, *H*, from overloads. The pilot light, *K*, indicated when the relay, *J*, was closed. The control leads from the relay were connected to the U-tube, *C*, by mercury wells around tungsten wires sealed through glass. This U-tube was used as a safety device to protect the column from running without the liquid reflux which was necessary to lubricate the bearings. Stopcocks *A* and *B* were connected to the vacuum line, and when the operating pressure was reached, *A* was shut off. The relay then was set normally closed. This safety device cut off the motor when the pressure went above a certain value. Since a pressure increase of a few millimeters could almost completely stop vaporization in the kettle, this safety device was of utmost importance. The throughput controller previously mentioned was also a valuable protection to the bearings. It kept the throughput constant during transitions from the boiling point of one fraction to a higher boiling fraction or provided the pot with extra heat if a heat loss developed anywhere in the column.

The revolutions per minute of the rotor were indicated by a Weston alternating current voltmeter calibrated for revolutions per minute from 0 to 10,000 in units of 100 r.p.m. The alternating current potential proportional to the revolutions per minute was generated by a Weston alternating current tachometer generator, which was mounted above the top of the rotor shaft and was connected to it with a piece of heavy rubber tubing. Another tachometer drive consisted of a rubber-edged pulley on the generator, which was driven by the outside edge of the column V-belt. This arrangement worked satisfactorily, but required calibration.

Pressure Control. The pressure in the system was lowered by a Cenco Hy-Vac pump and was regulated by a sulfuric acid–diethylene glycol manostat. When the relay was bleeding air into the system it turned off the pilot light on the control panel. Thus, satisfactory operation of the vacuum system was indicated by the blinking of the pilot light. The pressure in the system could be read in units of 0.1 mm. of mercury, up to 100 mm., by a Zimmerli gage. Back pressure was determined by a U-tube containing mercury. The best arrangement found was to use, as the back-pressure line from the kettle, a large diameter, long glass tube with a large stopcock close to the kettle. This tube acted as a vapor interface chamber and as a condenser. It had sufficient volume to register the correct value of the back pressure over the range of pressures encountered, which was from 0 to 25 mm. of mercury. Further diffusion into this line was retarded by a capillary tube and a small rubber tubing used as the back-pressure line to the control panel.

Distillation Head. The distillation head used (Figure 9) was a modified Collins and Lantz (*4*) type and had a relatively high throughput capacity.

The vapor passage had a minimum cross section of 0.5 square inch, which was larger than the smallest cross sectional area of 0.41 square inch in the annular space in the column. The head had an outside diameter of 3 inches and was 8 inches long, exclusive of the 50/30 glass semiball joint used to connect the head to the column. The condenser was attached with a 40/50 standard taper joint *A*. A ground-glass taper for a standard taper thermometer or a thermocouple well was provided in the side arm, *B*. The product condenser, not shown, was connected with a 35/20 semiball joint. Expansion bellows were provided in the inside wall as well as the outside wall of the vacuum jacket.

The take-off valve, *C*, was controlled by a solenoid connected by rod, *D*, and located above the condenser. The solenoid was

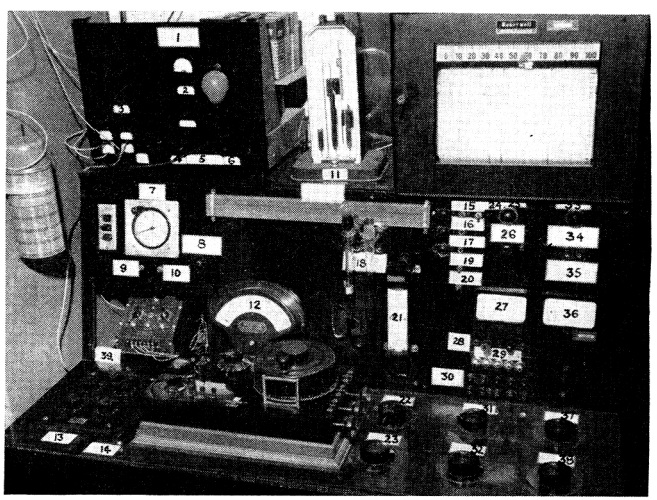

Figure 10. Control panel

1. Throughput controller
2. Ammeter
3. Bridge phase controller
4. Bridge transformer
5. Filament heater switch
6. Magnetic switches
7. Timer
8. Thermocouple selector switch
9. Still head solenoid switch
10. Timer switch
11. Zimmerli gage
12. Tachometer
13. Decade resistance
14. Manostat control dial
15. Manostat switch
16. Vacuum pump switch
17. Water pump switch
18. Back-pressure manometer
19. Galvanometer light switch
20. Zimmerli gage light switch
21. Flowmeter
22. Powerstat, motor speed
23. Powerstat, middle column heater
24. Motor safety circuit manual contro
25. Motor circuit pilot light
26. Motor switch
27. Voltmeter
28. Ammeter circuit selectors
29. Jack positions
30. Heater switches and fuses
31. Powerstat, cross arm heater
32. Powerstat, buttom column heater
33. Main power pilot light
34. Main magnetic switch button
35. Magnetic switch button
36. Ammeter
37. Powerstat, upper column heater
38. Powerstat, kettle heater
39. Reflux ration controller

operated by an automatic timing device. These last two parts are not shown in the figure.

The possibility of leakage around this valve or contamination of the vapor sample is eliminated by use of a female 28/15 semiball joint as the valve plunger. This joint seats on a male 28/15 semiball joint, as shown in the figure. Seepage between the two ground surfaces is retarded because of gravity and the greater area of contact between the two parts of the ball joint. Leakage of drops clinging to the plunger into the product line is prevented because the female semiball joint acts as an umbrella over the product line port.

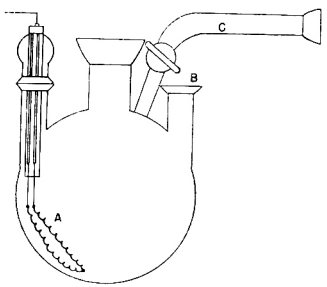

Figure 11. Test kettle

A. Heater
B. Ground-glass joint for sample thief
C. To manometer

An advantage in the fabrication of this head is that these standard semiball joints are commercially available and do not require hand grinding. The 28/15 female semiball joint will not quite pass through the 40/50 female standard taper at the top of the head; some of its edge must be removed by grinding. When the male 40/50 standard taper on the condenser is inserted, it provides a seat for the plunger when it is lifted by the solenoid at the top of the condenser. This shuts off the main condenser at the same time the product take-off is opened. The narrow ground-glass surfaces of contact, when the valve is lifted, are indicated by the shaded areas in the figure.

The maximum throughput which this head can handle has not been determined, but it has operated satisfactorily at a rate of 8 liters per hour.

The head take-off was operated by a repeat cycle timer obtained from the G. C. Wilson Co., Huntington, W. Va.

PERFORMANCE OF COLUMN

The column was operated by means of the control panel shown in Figure 10. The procedure used to place the column under test, after charging the kettle, was as follows.

The 110-volt line was connected to the terminal blocks by pressing the magnetic switch button, 35. The heater switches, 30, were then turned on. After reflux had been established at the head, rotation was started by turning on the motor switch, 26, and the manual relay switch, 24. The speed was adjusted by the powerstat, 22, and indicated by the tachometer, 12. When the speed of rotation was set at the desired value, the temperatures of the column heaters were lowered until the throughput reached a chosen value, as indicated by the thermocouples in the inlet and outlet of the condenser. These thermocouples could be read by turning the selector switch, 8, to position 12. If the throughput controller, 1, described above, was used, it was turned on by a switch not labeled. The filament heater switch, 5, was turned on for 30 minutes before the bridge transformer, 4, or magnetic switches, 6, were closed.

When these were turned on, the throughput was adjusted by the decade resistance, 13. The amperes supplied to the internal heater were measured by the ammeter, 2. The amperage was limited to 3.0 amperes by a variable resistance located inside the panel. When it was desired to commence automatic take-off, the repeat cycle timer was turned on by switch 10 and adjusted to the proper rate using the timer, 7. Either the off cycle or the on cycle could be timed by use of the upper of the two switches on the left of the timer. The lower switch connected the timer to an extra outlet below the switches. After the time cycles were adjusted, switch 9 was turned to allow the power to go to the solenoid which lifted the vapor dividing valve.

Test Apparatus. The vapor dividing head, described above, proved satisfactory for the withdrawal of small samples of distillate. The samples were collected in 10-ml. graduated tubes by applying power to the solenoid lift valve for a sufficient time (measured by a timer) to obtain a sample of the desired size, generally about 1 ml.

The test kettle with accessories is shown in Figure 11. It was equipped with an internal heater, *A*, which was powered by the throughput controller described above. The internal heater was connected to the 3-liter, three-necked, round-bottomed flask by a 28/15 semiball joint.

The back-pressure manometer line was connected to the tube, *C*, with a 28/15 semi-ball joint. The tube, *C*, could be closed by a stopcock. The bores of the tube and the stopcock were large enough to allow drainage of the test mixture which condensed in the larger diameter, horizontal vapor interface chamber.

A combination kettle sampling thief (Figure 12) and thermocouple well was inserted at *B*. The sample thief could be used at atmospheric and reduced pressures, and has the advantages of providing representative and uncontaminated samples.

The sampling line, *A* (Figure 12), was made from thick-walled capillary tubing to provide strength and to reduce holdup of liquid in the tube. When samples were not being withdrawn, the sampling tube was shut off by the stopcock, *B*. The sample was collected in a graduate centrifuge tube, *D*. This receiver was connected to the sampling tube by a 19/38 standard taper joint attached to the splash chamber, *C*, which was large enough to prevent the liquid being sampled from splashing or boiling into the vacuum line.

A kettle sample was withdrawn by closing stopcock *B* and connecting one arm, *F*, of the two-way stopcock, *E*, to a pressure sufficiently lower than that the system to permit the liquid to be forced into the sample receiver. The other arm, *G*, was closed to the atmosphere. With stopcock *E* opened to the lower pressure arm, *F*, and the receiver in place, stopcock *B* was opened to allow the kettle liquid to fill the receiver. When the receiver was full and the system below atmospheric pressure, stopcock *E* was opened to the atmosphere through arm *G*. By this procedure the liquid was forced into and out of the sampling tube several times before a sample was taken for analysis. The source of the lower pressure may be a vacuum pump when the condenser pressures are less than 50 mm. of mercury, or a water aspirator when the pressures are greater than 50 mm. of mercury. When the system was at atmospheric pressure, a rubber bulb was attached at *F* and used for alternate suction and pressure.

The alternate filling and emptying of the receiver assured a representative sample uncontaminated by stopcock lubricant, by small amounts of impurities which may have been in the receiver, or by previous samples left in the sampling line. The sampling line reaches almost to the bottom of the receiver, *D*, and thus made it possible to force all but a small amount of liquid back into the kettle after the test lines and the receiver were washed out with the larger samples.

H is a semiball joint which fits into the kettle, and *I* is a thermocouple well which is incorporated in this particular sampling device.

Dibutyl phthalate was used in the thermocouple well as a heat

exchange medium. The temperature in the well was indicated by use of a thermocouple.

OPERATING VARIABLES

Throughput. One of the important variables is the throughput, for which there are several definitions. One states, "The throughput, boilup rate, or vapor velocity, is the rate at which the vapor is passing up the column and is usually expressed in terms of the quantity of liquid equivalent to the vapor passing up to the column per unit time" (*11*). Another states, "The vapor velocity may be expressed either as unit weight per unit time of material reaching the top of the column, or as unit distance per unit time" (*8*). These definitions may be consistent and sufficient if the column is being operated under adiabatic conditions. However, when adiabatic conditions are not maintained, the various terms quoted above do not have the same meaning, as the boilup rate would not necessarily be the same as the condensation rate at the head condenser.

To describe clearly nonadiabatic operating conditions, both the boilup rate and the condensation rate at the head should be stated.

In this paper throughput is defined as the moles of material per unit time passing up the column when the operating conditions of the column were adjusted so that the boilup rate was equal to the condensation rate at the head condenser. This definition permits the most consistent correlation of throughput with the other variables. The definition of throughput is of particular importance in describing the operation of the rotating column for two reasons. First, the throughput has a critical effect of the efficiency and other characteristics of the column. Secondly, the rotating concentric tube column differs from previously reported types of distillation columns in that the rotation produces a large amount of heat in the column. The amount of heat released by the rotation depends on the viscosity and quantity of the material passing through the column as well as on the friction in the shaft bearings. The heat produced by these effects is equivalent in this column approximately to an increase in condensation rate at the head of 500 ml. per hour of the test mixture per 1000 r.p.m. For example, at 5000 r.p.m., with a boilup rate of 500 ml. per hour at the kettle, over 2000 ml. per hour would be condensing at the head if the column heating jackets were maintained at the boiling temperature of the reflux. Thus, the conventional methods of operating a column adiabatically by surrounding the column with vacuum jackets, heavy insulation, or air heated to the column temperature, result in wide deviation from adiabaticity in the rotating column.

In practice the throughput was controlled as follows. The heat loss in the kettle charged with distilland was determined by plotting the kettle temperature against the amperes passing through the pot heater to produce that temperature. The horizontal portion of the resulting curve indicated that the boiling point was reached and that additional heat went into vaporization of the charge. The heat loss in watts was calculated by multiplying the amperes at the break in the curve by the voltage at that amperage. This wattage was then added to the wattage required to vaporize the material in the still at the desired rate.

The following heats of vaporization were used in this investigation (*5*): *n*-Heptane at the normal boiling point has a value of 75.6 cal. per gram, and at 25° C., 87.2 cal. per gram. Methylcyclohexane has a heat of vaporization at the normal boiling point of 77.2 cal. per gram, and at 25° C., 86.1 cal. per gram. The column heaters were then adjusted to provide the same condensation rate in the head condenser as the boilup rate in the kettle. At 4000 r.p.m. this required that the column heaters be operated at about 10° C. below the kettle temperature. Thus, at the higher revolution speeds, the heating jackets served essentially as a controlled heat leak to remove the heat generated by rotation. Once the column jacket temperatures had been adjusted for a particular rotation speed, the throughput could be controlled by adding or subtracting the calculated wattage from the kettle heater. This indicates that the over-all heat absorbed or lost in the column was reduced to a very low value, and that the column was operated under conditions which simulated adiabatic conditions in so far as the liquid and vapor were concerned.

Pressure Drop. The pressure drop in the rotating column is principally related to the revolutions per minute and the throughput. However, the pressure drop in the rotating column did not give a satisfactory indication of throughput because of its low values and the difficulty of measurement. It is believed that this method of estimating throughput is unsatisfactory for any column in which the pressure drop is small, such as the spinning band or spiral screen types. Because the pressure drop was affected by more than one variable, it is difficult to attach a useful significance to it. The principal value of its measurement in the rotating column was in indicating floods. When the pressure drop exceeded about 25 mm., a flood soon became evident at the head.

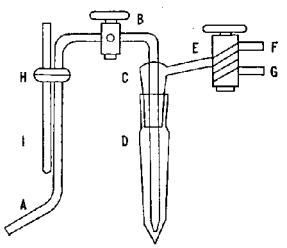

Figure 12. Kettle sampling thief

A. Capillary sampling tube
B. Stopcock
C. Splash chamber
D. Graduated receiver
E. 2-way stopcock
F. To low pressure source
G. To atmosphere
H. Ground-glass joint
I. Thermometer well

Theoretical Plates. The theoretical plates in the rotating column depend principally on the throughput, the speed of rotation, and the condenser pressure. It is suggested that at total reflux the highest possible theoretical platage may not necessarily be obtained by operating the column according to the definition of throughput given above. When the column was operated according to the definition, and then either the boilup rate or head condensation rate was lowered with the other rate held constant, there were indications that the number of theoretical plates increased. Also, there were indications that when either the boilup rate or the condensation rate was raised with the other rate held constant, the theoretical plates decreased. Whether there would be a similar effect at finite reflux is uncertain.

Holdup. The static or nondrainable holdup is expressed as the quantity of *n*-heptane which remained in the column after refluxing a mixture of *n*-heptane and dibutyl sebacate at 1 atm. pressure, then allowing the column to drain for 1 hour.

The operating holdup was determined at atmospheric pressure at 0, 2000, and 4000 r.p.m., by using the above mixture. Analyses of the composition of the mixtures in the kettle, before and during distillation at total reflux, were made by use of the refractometer. Dibutyl sebacate is commercially available and extremely nonvolatile. It may be readily purified by taking the center cut when distilled at reduced pressures.

The data for the relation between composition and refractive index of the *n*-heptane–dibutyl sebacate mixture are as follows:

Dibutyl Sebacate, Wt. %	n_D^{25}
0.0	1.3852
15.21	1.3912
25.64	1.3971
36.86	1.4028
47.92	1.4084
57.46	1.4140
67.20	1.4193
75.55	1.4241
84.35	1.4294
92.24	1.4343
100	1.4398

It is believed that this mixture has certain advantages over some mixtures previously used. When stearic acid is used as the nonvolatile component, the analysis is ordinarily carried out by titration with a base or by evaporation and weighing of the residue. These methods require at least 5-ml. samples and are time consuming. When rosin oils are used, the mixtures are analyzed by refractive index measurements. However, inasmuch as refractive index of rosin oils varies with each batch, no general relationship between composition and refractive index can be established.

The holdup data are given in Table I.

Table I. Holdup of Rotating Column Using *n*-Heptane–Dibutyl Sebacate Test Mixture

Throughput, Moles/Hr.	R.P.M.	Holdup, Moles *n*-Heptane
0.0	0	0.691
3.4	0	1.05
6.7	0	1.14
6.7	2000	0.947
6.7	4000	0.964
13.4	0	1.20

Rotation was found to reduce holdup but the speed of rotation seemed to have little effect; 2000 and 4000 r.p.m. gave almost identical values. The holdup at zero throughput is the static hold and seems to account for a large portion of the operating holdup. This is probably due in part to the cross arm. Because of space limitations, the flexible joint in the cross arm had to be mounted almost horizontally, and thus, did not drain so well as if it had been mounted vertically.

COLUMN TESTING

The test mixture used was *n*-heptane and methylcyclohexane. The methylcyclohexane had a refractive index at 25° C. of 1.4206 and was obtained from the Dow Chemical Co. The *n*-heptane had a refractive index of 1.3852 and was obtained from the Phillips Petroleum Co. This mixture was analyzed by refractive index using the data of Hawkins and Brent (*7*).

Approximately 1-ml. samples were taken from the head and the pot until successive samples checked. At least 1 hour was allowed between successive checks, and several hours were allowed at higher platages. Equilibrium was established in about 2 hours at the higher throughputs. However, when the column was operated under the conditions giving the highest platages, several days were required.

For this test the column was brought to equilibrium at the highest rotation speed and the lowest throughput to be studied. After data at this point were obtained, the throughput was increased to the next higher value. When all throughputs at this rotation speed had been studied, the speed was changed to the next lower value and the throughput established at the lowest value to be studied at that speed of rotation. The procedure was continued to the lowest speed of rotation and the highest throughput.

Once the mole fractions of *n*-heptane at the head and the kettle were obtained from the refractive indices, the theoretical plates at total reflux were calculated by use of the Fenske equation in the form:

$$n = \frac{1}{\log \alpha} \log \frac{X_n(1 - X_s)}{X_s(1 - X_n)}$$

where n = the number of theoretical plates, α = the relative volatility, X_n = mole fraction of more volatile component at the head, and X_s = mole fraction of more volatile component in the kettle.

The kettle charge was approximately 1.5 liters in a 3-liter kettle. This size charge was used to minimize the effect of possible contamination of the charge by the ground-joint lubricant or rotating seal oil, and to allow the use of small mole fractions of the more volatile component. This last factor is important because at higher values of platage the holdup contained a large quantity of *n*-heptane. To ensure against possible errors due to contamination of the kettle with the nonvolatile joint lubricant or seal oil, kettle samples of about 10 ml. were taken at intervals and distilled in a small distilling apparatus. The sample volume was noted before and after distillation to check on loss of sample. If the refractive index of the distilled sample differed by more than one or two in the fourth decimal place from that of the original sample, the kettle was cleaned and recharged. Otherwise, the corrected index was used. Generally the kettle composition remained essentially constant for long periods of time.

It was necessary to use low concentrations of *n*-heptane in the kettle in order to show the higher platages. At 1 mole % the head composition reached 97.5 mole % for 5000 r.p.m. and 3.4 moles per hour. If a much higher kettle composition was used, the head sample would be almost pure *n*-heptane and calculate infinite platage.

RESULTS AND DISCUSSION

Atmospheric Pressure. In general, the results of the tests at atmospheric pressure parallel those obtained by Willingham, Sedlak, Rossini, and Westhaver (*12*).

Figure 13 shows the effect of the rotation on the efficiency of the column at several values of throughput. As was noted at the National Bureau of Standards, the efficiency increased relatively slowly at first with increasing speed of rotation. According to the theoretical work of Willingham, Sedlak, Rossini, and Westhaver (*12*), the high efficiency of the rotating concentric tube column is due to the increase in the vapor diffusion coefficient resulting from the change from laminar flow, which is characteristic of stationary concentric tube columns, to turbulent flow. After the onset of turbulence, the rotation had a marked bene-

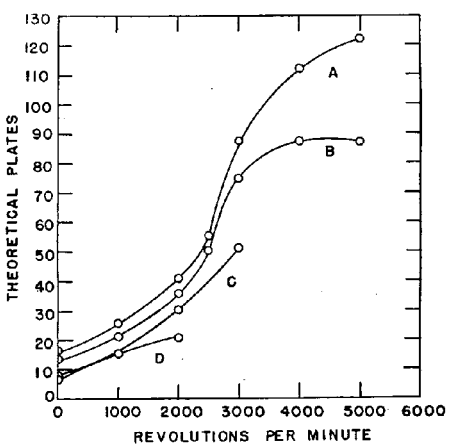

Figure 13. Theoretical plates *vs.* speed of rotation

Condenser pressure, 760 mm. of mercury; test mixture, *n*-heptane–methylcyclohexane
Moles per hour:
A. 3.4 *C.* 13.4
B. 6.7 *D.* 20.1

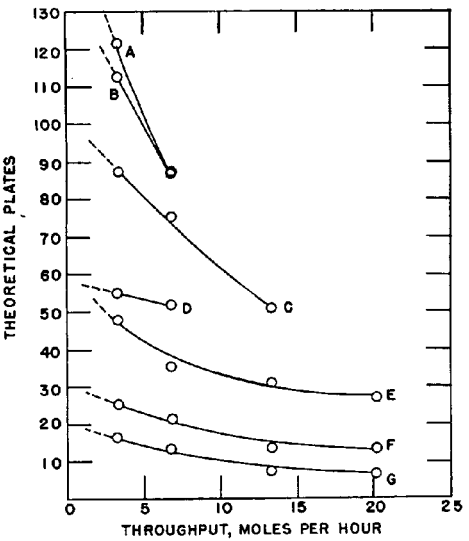

Figure 14. Theoretical plates *vs.* throughput

Condenser pressure, 760 mm. of mercury; test mixture, *n*-heptane–methylcyclohexane
Revolutions per minute:
A. 5000 *D.* 2500
B. 4000 *E.* 2000
C. 3000 *F.* 1000
G. 0.0

ficial effect on efficiency. No effort was made in this investigation to determine exactly the rate of rotation at the onset of turbulent flow, but Figure 13 suggests that this change occurs between 1500 and 2000 r.p.m. This is to be expected, because the present rotating column produces the same peripheral velocity at 1725 r.p.m. as the Bureau of Standards' column produced at 2300 r.p.m., the point of onset of turbulence determined previously (*12*).

It is difficult to compare the performance of the present rotating column with the Bureau of Standards' column, which was run at higher throughputs using a different test mixture and under different conditions of operation. Willingham and coworkers measured throughput only by the heat input to the kettle corrected for heat loss, and operated the column heaters at the reflux temperatures at all speeds of rotation. The throughput is expressed in the present work as moles per hour because moles per hour is more nearly proportional to the vapor velocity than milliliters per hour or grams per hour, and hence, should give a better correlation with the results of other test mixtures. For purposes of rough comparison with the Bureau of Standards' work, 6.7 moles of the present test mixture is approximately 1 liter of liquid. In general, the present rotating column has somewhat lower efficiency than that of the Bureau of Standards' column after the data of the latter were extrapolated and adjusted for the difference in dimensions of the two columns. Many of the points reported (*12*) at the higher throughputs were impossible to obtain at the higher rotation speeds used in this study because of flooding, which results from the increased back pressure in the present column. However, when the present column was operated with the column heating jackets at the reflux temperatures, it was possible to condense large amounts of material at the head with high speeds of rotation, and to obtain platages similar to the extrapolated National Bureau of Standards' values. For example, with less than 500 ml. per hour vaporizing in the kettle, about 2400 ml. per hour condensed at the head at 4000 r.p.m. These conditions produced 76.8 theoretical plates. However, the platages obtained with equal vaporization and condensation rates are definitely lower than the extrapolated National Bureau of Standards' data, especially considering the fact that the peripheral velocity is increased one third over that of the Bureau's column due to the increased diameter of the rotor. Whether this platage, lower than anticipated from these extrapolation values, is due to the difference in operation, to possible difference in the surface of the rotor, to inherent lack of accuracy in extrapolating data from a 23-inch rectifying section to a 50-inch section, or to unknown factors, is a question which will require extensive investigation before it is resolved.

Because the diameter of the present column was one third greater than that of the Bureau's column, the peripheral velocity at 5000 r.p.m. was equivalent to the latter at 6667 r.p.m. Several interesting conclusions may be drawn from the extended data.

The initial rapid increase in efficiency with increasing rotation rate, which comes after the onset of turbulence, does not continue indefinitely, but appears to approach a maximum value at high speeds. Curves *A* and *B*, Figure 13, indicate that there is a different optimum speed of rotation for each value of throughput, and that the optimum speed is higher the lower the throughput.

The effect of throughput on efficiency at constant speeds of rotation is indicated in Figure 14. This plot shows the marked effect of throughput on platage as was found at the Bureau of Standards. The effect of throughput on efficiency is much greater at the higher speeds of rotation. The meeting of curves *A* and *B* verifies the last conclusion in the above paragraph. The reproducibility of the platage values is estimated to be within 5%. For example, the platage at 3.4 moles per hour was rechecked after a number of other runs at atmospheric pressure and reduced pressures. The column had been dismantled for maintenance between these checks. The two values obtained were 112.2 and 111.8.

The effect of speed of rotation on pressure drop at several values of throughput is shown in Figure 15. This plot shows that, at the lower values of throughput, the pressure drop is almost independent of speed of rotation, but at higher throughputs, the pressure drop is critically affected. The effect of throughput at several speeds of rotation is shown in Figure 16. As also shown in the previous figure, the flood point is accompanied by a sharp increase in pressure drop.

Reduced Pressure. The present vacuum-sealing system al-

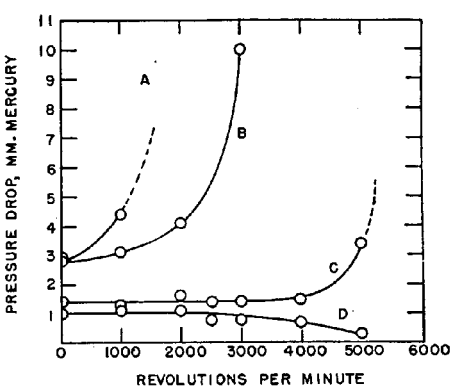

Figure 15. Pressure drop *vs.* revolutions per minute

Condenser pressure, 760 mm. of mercury
Moles per hour:
A. 20.1 *C.* 6.7
B. 13.4 *D.* 3.4

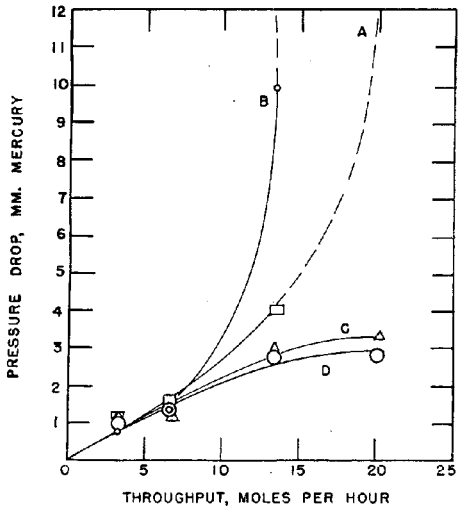

Figure 16. Pressure drop *vs.* throughput

Condenser pressure, 760 mm. of mercury; test mixture, *n*-heptane–methylcyclohexane
Revolutions per minute:
A. 2000 *C.* 1000
B. 3000 *D.* 0.0

lowed rotation at speeds up to 6000 r.p.m. for indefinite periods of time while maintaining a pressure of about 5 mm. of mercury.

Vacuum operation strongly brings out the difference in operating characteristics between the rotating concentric tube distillation column and other previously reported types of distilling columns. This is because at reduced pressures the boiling point of the test mixture was greatly reduced and, as this lowered the differential between the boiling point and room temperature, the excess heat generated by rotation was more difficult to remove. For example, with ethylbenzene and chlorobenzene test mixture at 20 mm., it was impossible at 4000 r.p.m. to obtain a throughput lower than 2 liters per hour, even though there was no heat applied to the column heaters. Even after the kettle heater was shut off for 0.5 hour, the head reflux stayed at 2 liters per hour, the sustaining heat being obtained only from rotation. At higher pressures it was necessary to remove all insulation around the column heating jacket, leaving the sheet metal tube and its covering of asbestos paper as the only column insulation. Operation at condenser pressures lower than used here would have necessitated an arrangement such as installing pipe fittings in the sheet metal tube and blowing cold compressed air through the spaces between the column and the sheet metal tube.

A study was made at 200 mm. pressure of the relation between platage, throughput, and speed of rotation, using the test mixture *n*-heptane–methylcyclohexane. The results obtained at this pressure are shown in Figures 17 and 18. The platages obtained at 200 mm. showed a marked decrease from the values obtained at 760 mm. This is possibly due to either decreased turbulence or increased vertical back diffusion.

It appears that the platage cannot be increased much beyond 35 by increasing the rotation speed. This is about one third the platage obtained at 760 mm. Rotation seems to have the same general effect at 200 mm. of mercury as at atmospheric pressure, but to a lesser degree. The effect of throughput on platage is the same as at atmospheric pressure, but curve *A*, Figure 18, at 4000 r.p.m. again shows an apparent limiting platage. Although the pressure drops at reduced pressures were about the same as at atmospheric pressure, it seemed to be possible to have larger throughputs at the high speeds of rotation under reduced pressure than at 760 mm. For example, a point was obtained at 13.4 moles per hour at 4000 r.p.m. at 200 mm., while at 760 mm. pressure, a flood resulted at this throughput. It was more difficult to obtain reproducible results at reduced pressures than at atmospheric pressure, but generally points were reproducible to within 10%.

In order to show the effect of various values of condenser pressure on platage, points were obtained at 6.7 moles per hour and 3000 r.p.m. at 200, 400, 600, and 760 mm. The data for these values show a continuous decrease in platage with decreasing pressure. It was impossible to obtain data at much lower pressures due to the heat dissipation problem.

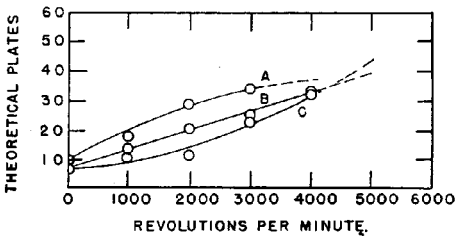

Figure 17. Theoretical plates *vs.* revolutions per minute

Condenser pressure, 200 mm. of mercury; test mixture, *n*-heptane–methylcyclohexane
Moles per hour:
A. 3.4 *B.* 6.7
C. 13.4

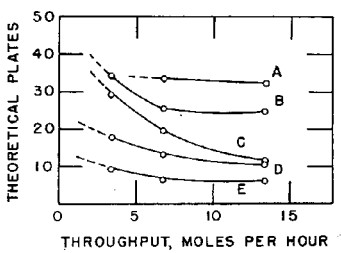

Figure 18. Theoretical plates *vs.* throughput

Condenser pressure, 200 mm. of mercury; test mixture, *n*-heptane–methylcyclohexane
Revolutions per minute:
A. 4000 *C.* 2000
B. 3000 *D.* 1000
E. 0.0

Because the relative volatility of the *n*-heptane–methylcyclohexane mixture changes with pressure, it was necessary to take this into consideration when calculating the platages at the various pressures. The relative volatilities used were calculated from the Antoine equations given by Jordan (*8*), and check the values given by Hawkins and Brent (*7*).

ACKNOWLEDGMENT

The authors wish to express their appreciation for the assistance of Calvin J. Workinger, Edward A. Warshyk, and Harold E. Crockford in the construction and maintenance of the column.

They also extend special thanks to the Globe Steel Tubes Co., who donated the stainless steel tubes through Elmer Gammeter.

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RECEIVED for review August 5, 1955. Accepted June 9, 1956.

Rapid Method for Simultaneous Determination of Sulfur and Phosphorus in Petroleum Products

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An extension of the high temperature combustion technique permits the rapid determination of sulfur and phosphorus in the same sample in an elapsed time of about 20 minutes. The sample, mixed with zinc oxide, is burned in a high temperature unit. The sulfur dioxide evolved is absorbed in an acid-iodide solution and the sulfur determined by immediate titration with potassium iodate. The residue of zinc phosphates in the combustion boat is dissolved and the phosphorus determined colorimetrically as molybdivanado phosphoric acid. The estimated standard deviation (σ) of the phosphorus test is about 0.02 above the 0.1% level and 0.003 below the 0.1% level.

THE trend toward continuous flow processes in many industries, including the petroleum industry, has placed heavy demands on the analytical chemist. Because it is not feasible to control a continuous process with a lengthy analytical procedure, it has become necessary to modify existing procedures and develop new ones that can be completed in a relatively short time. The usual determination of either sulfur or phosphorus requires a lengthy ashing or combustion step followed by a time-consuming precipitation or color development step. Accordingly, some method that would eliminate either or both of these steps was needed.

For many years the metal industries have been using high temperature combustion techniques for determining sulfur (*1*, *2*, *5*, *7–9*), but only recently has this method been used for organic materials such as petroleum products. Inasmuch as the determination of sulfur by high temperature combustion has been in routine use in this laboratory for several years, no extensive experimental work was done during this investigation. However, because the sulfur determination is an integral part of the phosphorus analysis, a description of the method is included here.

APPARATUS

A resistance furnace capable of producing temperatures in excess of 2300° F. can be used. With suitable modifications, it should be possible to use a high frequency induction-type furnace (*6*). General analytical laboratory facilities, including a spectrophotometer and gas absorbers of the type shown in Figure 1, are also required.

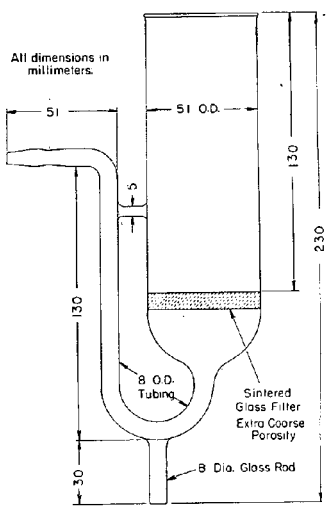

Figure 1. Modified absorber for use with high temperature combustion apparatus

REAGENTS

All reagents conformed to specifications established by the Committee on Analytical Reagents of the AMERICAN CHEMICAL SOCIETY.

Sulfur Determination. Zinc oxide.

Hydrochloric acid solution. Dilute 30 ml. of concentrated acid to 2 liters with distilled water.

Starch-iodide solution. Macerate 4.5 grams of soluble starch in 12 ml. of hot distilled water. Cool and add 7.5 grams of potassium iodide and dilute to 500 ml.

Potassium iodate solutions. Dissolve 1.1125 grams of dried reagent in distilled water and dilute to 1 liter. One milliliter of this solution is equivalent to about 0.5 mg. of sulfur. Pipet 200 ml. of this solution into a 1-liter volumetric flask and dilute to the mark; 1 ml. is equivalent to about 0.1 mg. of sulfur.

Table I. Determination of Phosphorus

Sample No.	Phosphorus Type	% Phosphorus Caled.	% Phosphorus Found[a]	% Deviation from Caled.
A	Phosphate	0.582	0.554	−5.05
B	Phosphite	0.973	0.937	−3.84
C	Phosphine	0.310	0.295	−5.08

[a] Average of three results.

Table II. Determination of Phosphorus in Known Blends

Type Compound in Blend	Weight % Theory	Found	Diff.
Phosphite	0.973	0.932	−0.041
		0.940	−0.033
		0.883	−0.090
		0.934	−0.039
		0.839	−0.134
		Av. 0.906	
Phosphate	0.903	0.824	−0.079
		0.862	−0.041
		0.844	−0.059
		0.862	−0.041
		0.852	−0.051
		Av. 0.849	
Phosphite	0.819	0.780	−0.039
		0.810	−0.009
		0.821	+0.002
		0.783	−0.036
		Av. 0.799	
Phosphite	0.804	0.758	−0.046
		0.762	−0.042
		0.734	−0.070
		0.708	−0.096
		0.745	−0.059
		Av. 0.741	
Phosphate	0.582	0.573	−0.009
		0.600	+0.018
		0.572	−0.010
		0.554	−0.028
		0.573	−0.009
		Av. 0.574	
Phosphate	0.533	0.510	−0.023
		0.495	−0.038
		0.525	−0.008
		0.506	−0.027
		0.519	−0.014
		Av. 0.511	
Phosphite	0.349	0.303	−0.046
		0.310	−0.039
		0.333	−0.016
		0.376	−0.027
		0.317	−0.032
		Av. 0.328	
Phosphate	0.115	0.110	−0.005
		0.115	0.000
		0.108	−0.007
		0.116	+0.001
		0.113	−0.002
		Av. 0.112	
Phosphate	0.0624	0.0559	−0.0065
		0.0610	−0.0014
		0.0552	−0.0072
		0.0568	−0.0056
		0.0529	−0.0095
		Av. 0.0564	
Phosphine	0.319	0.274	−0.045
		0.252	−0.069
		0.261	−0.058
		0.284	−0.035
		Av. 0.268	
Phosphine	0.596	0.503	−0.093
		0.514	−0.082
		0.501	−0.095
		0.518	−0.078
		Av. 0.509	

Phosphorus Determination. Ammonium molybdate solution. Dissolve 50 grams of ammonium heptamolybdate $[(NH_4)_6Mo_7O_{24}.4H_2O]$ in warm water and dilute to 1 liter. Filter before using.

Ammonium vanadate solution. Dissolve 2.5 grams of ammonium metavanadate (NH_4VO_3) in 500 ml. of hot water, add 20 ml. of nitric acid (specific gravity, 1.42) and dilute to 1 liter.

Sulfuric acid, 1 to 4.

Standard phosphate solution (1 ml. = 0.1 mg. of phosphorus). Dissolve 0.4393 gram of monobasic potassium phosphate in water and dilute to 1 liter. For best work the salt should be twice recrystallized and vacuum dried before use.

EXPERIMENTAL

Preparation of Blends for Phosphorus Determination. Satisfactory blends of known phosphorus content were made by first preparing concentrates of pure phosphorus compounds in an oxygenated diluent (2-ethyl butyl Cellosolve) and then diluting further with a phosphorus-free mineral oil. These blends were used in the experimental work to develop the procedure for phosphorus. Blends were prepared in the above fashion because it was found that the pure compounds, when blended directly with a straight mineral oil, separated therefrom in a short time.

Determination of Operating Conditions. The data shown in Table I were obtained with a furnace temperature of 2400° F. and an oxygen flow of 2 liters per minute. These are the settings normally used for the sulfur determination. However, the results in Table I show that the phosphorus values obtained were slightly lower than theory. Accordingly, changes, which the authors' experience with sulfur determinations indicated were permissible, were made in both temperature and oxygen flow rate. Neither lowering nor raising the temperature 100° F. and the oxygen flow 0.5 liter per minute affected the results, so it was decided to continue the work at the standard sulfur operating conditions (2400° F., 2 liters of oxygen per minute).

Preparation of Calibration Curves. Inasmuch as the determination of phosphorus is carried out spectrophotometrically, it is necessary to prepare suitable calibration curves. These curves are prepared as described in "ASTM Standards on Petroleum Products and Lubricants" (*4*), except that all standard solutions are made to 125-ml. volume instead of the 100 ml. specified in the reference.

PROCEDURE

Preparation of Apparatus. Turn on current to the furnace and adjust the controls so that the temperature is 2400° F. Add 65 ml. of dilute hydrochloric acid and 2 ml. of starch-iodide solution to the absorber. Add 1 drop of standard potassium iodate. Turn on the oxygen supply and adjust the flow rate to 2 liters per minute.

Sample Preparation. Weigh an appropriate-sized sample (20 to 30 mg. for additive concentrates, 150 mg. for lubricating oils) into a small platinum boat (approximately 25 × 5 × 5 mm.) filled almost to capacity with zinc oxide. Add additional zinc oxide to cover the sample. Place the platinum boat in a larger ceramic boat, place this assembly into the combustion tube, then insert the oxygen delivery tube in the end of the combustion tube.

Sulfur Determination. When the sample starts to burn, the color of the solution in the absorber gradually fades as sulfur dioxide is evolved. Maintain the blue color by the addition of standard potassium iodate. When the evolution of sulfur dioxide has stopped, push the boat into a hotter zone of the furnace and continue the titration as before. There are three temperature zones in the furnace, each one hotter than the preceding one. The sample is moved farther into the tube and titration of the evolved sulfur dioxide is continued until there is no further change in the intensity of the blue color in the absorber. The amount of standard potassium iodate added after sample combustion is complete is a measure of the sulfur content of the sample.

Phosphorus Determination. When the combustion is complete, remove the boats from the combustion tube and drop the platinum boat (containing the residue from the combustion)

into a Kjeldahl flask of 100-ml. nominal capacity, but which has been calibrated and marked to contain 125 ml. Add 12 ml. of 1 to 4 sulfuric acid, place on a digestion rack, and boil until all the zinc oxide and phosphates have dissolved. Remove from the digestion rack and, while the solution is still hot, add 10 ml. of ammonium vanadate and 10 ml. of ammonium molybdate solutions. Dilute to the mark (125 ml.) and mix well. If the sample contains barium, place the colored solution in a centrifuge tube and centrifuge for 2 minutes before filling the color cuvette. Transfer some of this solution to an appropriate cuvette and read the per cent transmittance with a suitable spectrophotometer. A reagent blank carried through the above procedure is used to set the spectrophotometer on 100% transmittance. The weight of phosphorus in the sample is read from the previously prepared calibration curves.

Calculations.

$$\% \text{ sulfur} = \frac{(V - V_0) \times F \times 100}{W}$$

$$\% \text{ phosphorus} = \frac{M \times F' \times 100}{W}$$

where

V = ml. of potassium iodate used to titrate sample
V_0 = ml. of potassium iodate consumed on blank
F = titer of potassium iodate expressed as mg. of sulfur per ml. of iodate
M = mg. of phosphorus read from calibration curve
W = sample weight in mg.
F' = correction factor

DISCUSSION

Scope of Phosphorus Method. To determine the applicability of the test, a blend of each of three organic phosphorus compounds in mineral oil was prepared. Analysis of these blends, containing either a phosphate, a phosphite, or a phosphine, gave the results shown in Table I. The data show that the results are consistently lower than theoretical by 4 to 5%, indicating the possibility of obtaining good results if a firm correction factor could be established.

An interesting sidelight to these experiments is the observation that phosphines can apparently be analyzed by this rapid method with a recovery equal to that of other phosphorus compounds. This is not true with other combustion methods of analysis (*3*).

In order to determine the validity of the procedure, replicate determinations were made on several blends containing known amounts of phosphorus. The data given in Table II show that the method tends to give results which are somewhat low. At the present time the exact nature of the loss has not been established. There was no indication that the phosphorus had fused with the platinum boat to account for the low recovery. However, it is conceivable that the loss is caused by spattering, entrainment, or volatilization.

If the data are plotted (Figure 2), a straight line relationship is found to exist. Phosphines, phosphates, and phosphites fall on this line, indicating that all three type compounds can be determined by this method.

The least squares equation for this curve is

$$Y = 0.006 + 1.056\,X$$

where

X = observed per cent phosphorus
Y = best value for per cent phosphorus

The coefficient of correlation for this curve is 0.997, indicating that a high degree of confidence can be placed in the result ob-

Table III. Rapid Determination of Phosphorus and Sulfur in Lubricating Oils and Additives

Sample No.	Weight % Phosphorus Found: ASTM D 1091 (*4*)	Quick Method[a] Av.	Quick Method[a] Range
	Lubricating Oils		
1	0.008	0.009	0.006
2	0.046	0.045[b]	...
3	0.078	0.078	0.010
4	0.141	0.139	0.030
5	0.160	0.158	0.009
6	0.480	0.471	0.020
7	0.596	0.543	0.011
	Additive Concentrates		
8	0.960	0.932	0.00
9	0.978	0.960	0.080
10	1.31	1.31[b]	...
11	1.62	1.64	0.19
12	2.57	2.54	0.05
13	3.35	3.42	0.04
14	7.60	7.80	0.15

[a] Average of three determinations, corrected values.
[b] Single determination.

Table IV. Effect of Centrifuging on Phosphorus Determination

Sample	% Phosphorus Found[a]: Before centrifuging	After centrifuging	% Phosphorus, Theory
D	0.065	0.052	0.056
E	0.095	0.084	0.080
F	0.153	0.149	0.150

[a] Corrected values.

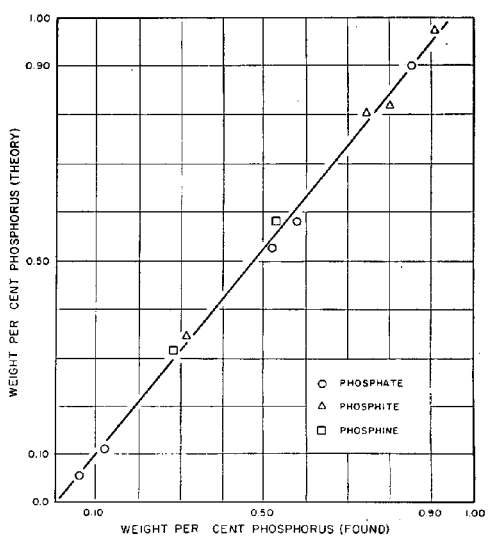

Figure 2. Ratio of quick method *vs.* theory for different types of phosphorus compounds

% theory = 0.006 + 1.056 (% found)
Correlation coefficient (r) = 0.997

tained by applying a correction factor to the observed value. The coefficient, 1.056, is the correction factor (F') to be applied to the observed phosphorus value. The constant (0.006) is smaller than the expected repeatability of the test and for all practical purposes can be ignored.

Several samples covering many types of materials and a wide range of phosphorus content were run by this technique and by a standard method of analysis (*4*). The data obtained are shown in Table III. The rapid method yields results which are in good agreement with those obtained by the conventional method.

Samples containing barium yield high results due to the presence of barium sulfate, which absorbs some of the light passing through the sample. This interference can be removed by centrifuging the sample. Table IV presents comparative data obtained when this type of sample is run with and without centrifuging.

Table V. Determination of Phosphorus and Sulfur in Same Sample

Sample No.	Phosphorus		Sulfur	
	Calcd.	Found[a]	Calcd.	Found
13A	2.59	2.40	2.95	2.94
14A	0.98	0.96	0.34	0.32
12A	2.58	2.58	2.89	2.83

[a] Corrected values.

To demonstrate that this procedure can be used to determine both sulfur and phosphorus in the same sample, blends containing known amounts of these elements were analyzed. The results (Table V) indicate that the method is capable of yielding accurate results for both elements.

PRECISION

Based on the data available at this time, the precision of these determinations appears to be as follows:

Element	Concentration Level (%)	Precision (2σ)
Sulfur	1.0+	0.11
	0.1 to 1.0	0.05
	0.05 to 0.1	0.02
Phosphorus	1.0+	0.12
	0.10 to 1.0	0.040
	0.05 to 0.10	0.006

ACKNOWLEDGMENT

The authors wish to express their thanks to E. R. Hartmann of the Esso Research and Engineering Co., Products Research Division, for his help in making many of the analyses during this investigation.

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RECEIVED for review May 2, 1956. Accepted July 12, 1956. Division of Analytical Chemistry, 129th meeting, ACS, Dallas, Tex., April 1956.

Determination of Beryllium in Titanium Alloys

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A colorimetric method for determining beryllium in titanium alloys has been developed, using 4-(*p*-nitrophenylazo)orcinol. The reagent dissolved in 0.1*N* sodium hydroxide forms a red-brown lake with beryllium in a 0.4*N* sodium hydroxide solution buffered with borate and citrate. The dye is nearly specific for beryllium. Titanium is held in solution with an excess of peroxide. Other interfering ions are held in solution as chelates of tetrasodium (ethylenedinitrilo)tetraacetate. The method is satisfactory for 0.25 to 1.0% beryllium in titanium alloys.

NO METHOD for the determination of beryllium in titanium has been published (*8*), and the Metallurgical Advisory Committee on Titanium, Panel on Methods of Analysis, has not created a Task Force on Beryllium. Existing methods for the detection and determination of this element in other materials were therefore reviewed, in order to find a method that could be adapted to titanium alloy containing 0.25 to 1.0% of beryllium.

Among those considered have been methods based on the following reagents which form colored complexes with beryllium: quinalizarin (*2*), curcumin (*3*), 4-(*p*-nitrophenylazo)orcinol (*9*), sulfosalicylic acid (*7*), solochrome cyanine (*10*), and aurin tricarboxylic acid (*5*, *6*). Of these reagents, 4-(*p*-nitrophenylazo)-orcinol was found to be best suited for use with titanium because it is nearly specific for beryllium. It is sensitive enough to detect small quantities of the element, it is not subject to many interferences, and the complex formed is stable for several hours.

Vinci (*9*) has used the reaction of Komarovskiĭ and Poluektov (*4*), in which 4-(*p*-nitrophenylazo)orcinol in 0.1*N* sodium hydroxide, when added to a moderately alkaline solution containing beryllium, forms a red lake.

Vinci noted that the absorption of light by this colored lake does not conform strictly to Beer's law. However, the relationship of light absorption to beryllium content can be fixed by careful control of pH, dye strength, temperature, and amount of other ions present. The pH can be regulated with a sodium borate-citrate buffer. Magnesium and zinc interfere by reacting with the dye. These metals and also the metals that form colored ions or precipitates in alkaline solution, such as copper, nickel,

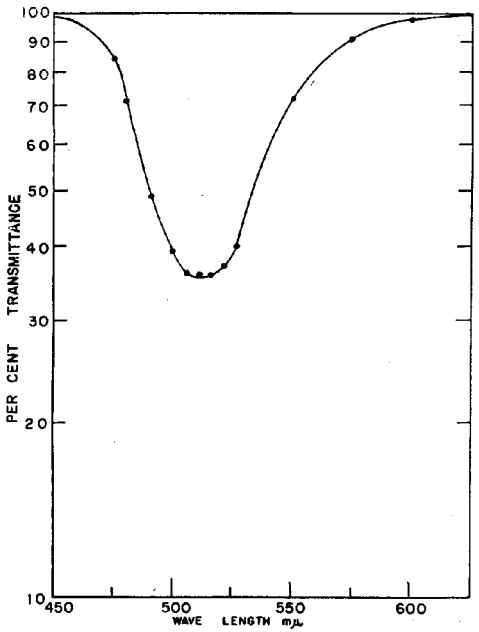

Figure 1. Transmittance curve

iron, and calcium, are rendered harmless by chelation with the tetrasodium salt of (ethylenedinitrilo)tetraacetic acid.

Titanium in relatively high concentrations as encountered in the analysis of titanium alloys must be prevented from precipitating in the alkaline solution. This has been accomplished by the combined use of tetrasodium (ethylenedinitrilo)tetraacetate and hydrogen peroxide. Without hydrogen peroxide the chelating agent will not prevent the hydrolysis of titanium in the alkaline solution when it is the major constituent of the sample. Titanium, vanadium, and molybdenum, which form colored complex ions with peroxide in acid solutions, do not form interfering colors in the buffered solution. The tetrasodium salt of (ethylenedinitrilo)tetraacetic acid was found to be more effective in alkaline solution than other complexing agents for preventing interference by titanium. Interference by magnesium and by the ordinary components of titanium alloys such as iron, chromium, and vanadium, which are colored or form precipitates, was also effectively eliminated by use of the tetrasodium salt.

REAGENTS

Standard beryllium solution, 0.1 mg. of beryllium per ml. Dissolve 1.966 grams of beryllium sulfate ($BeSO_4 \cdot 4H_2O$) in water and dilute to 1 liter.

Table I. Data for Calibration Curve

Number	Be, Mg./100 Ml.	% T
Blank	0.00	100.0
1	0.05	85.3
2	0.10	72.0
3	0.20	54.4
4	0.30	40.1
5	0.40	31.0
6	0.50	25.0
7	0.60	21.0
8	0.70	17.8

Buffer solution. Dissolve 116 grams of citric acid, 58.7 grams of anhydrous sodium borate, and 216 grams of sodium hydroxide in water and dilute to 1 liter.

Dye solution, 4-(*p*-nitrophenylazo)orcinol (Eastman Kodak P4414). Dissolve 0.150 gram of the dye in 500 ml. of 0.1*N* sodium hydroxide solution by stirring with a mechanical stirrer for 5 hours and filtering through an asbestos mat. Store in a red (low actinic) glass bottle. Renew this solution about once a month.

Chelating solution. Dilute one volume of a 47% water solution of tetrasodium (ethylenedinitrilo)tetraacetate (Versene Regular) with three volumes of distilled water (*1*).

Hydrogen peroxide, 3% solution.

Sodium hydroxide solution, 2.0*M*.

Hydrochloric acid, specific gravity 1.18.

Sulfuric acid, specific gravity 1.84.

PROCEDURE

Dissolve 5 grams of the sample in 1 + 1 hydrochloric acid or 20% sulfuric acid, cool, dilute to 100 ml., and take an aliquot containing 0.05 to 0.6 mg. of beryllium, not more than 5 mg. of magnesium, 20 mg. of calcium, 10 mg. of iron, 35 mg. of aluminum, and not more than 100 mg. of titanium.

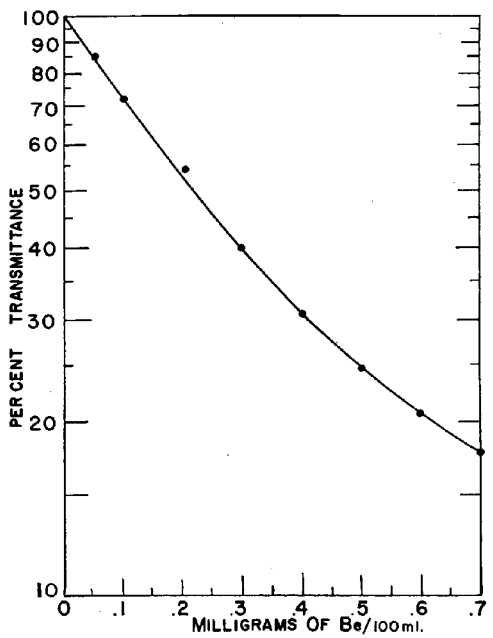

Figure 2. Calibration curve at wave length 515 mμ

Place in a 125-ml. Erlenmeyer flask and adjust the volume to approximately 15 ml. Add 15 ml. of 3% hydrogen peroxide and 5 ml. of the chelating solution. Adjust the pH to 5.5 using 2*N* sodium hydroxide or hydrochloric acid as needed. Let stand 5 minutes.

Add 10 ml. of buffer solution and let stand 5 minutes.

Transfer to a 100-ml. volumetric flask. Add exactly 10 ml. of the dye solution. Make up to 100 ml. with distilled water and let stand for 10 minutes.

Determine the per cent transmittance with a spectrophotometer adjusted to 100% transmittance with a reagent blank which does not contain titanium.

The transmittance curve, Figure 1, shows maximum absorbance near the wave length of 515 mμ. The samples are, therefore, read at 515 mμ, using a slit width of 0.01 mm. with the Beckman Model DU spectrophotometer.

Determine the beryllium content by reference to a previously constructed calibration curve.

PREPARATION OF CALIBRATION CURVE

The calibration curve was prepared by taking a series of aliquots of the standard beryllium solution, to cover the range 0.05 to 0.70 mg. of beryllium, and subjecting them to the procedure outlined (Table I). Cells having a light path of 10 mm. were used.

The curve was drawn by plotting % T against milligrams of beryllium on semilogarithmic paper (Figure 2).

DISCUSSION OF RESULTS

Five synthetic samples, three of which were made by additions of a standard beryllium solution to solutions of commercial alloys, were prepared and analyzed. The composition of the samples and results of analysis are shown in Tables II and III.

Table II. Synthetic Samples

Aliquot	Be Added, %	Be Found, %	Difference, %
	Synthetic 1. Ti 90%, Mo 2.0%, Cr. 2.0%, Al 6%		
A	0.07	0.076	+0.006
B	0.07	0.074	+0.004
C	0.70	0.684	−0.016
D	0.70	0.680	−0.020
	Synthetic 2. Ti 92%, Mo 2%, Al 4%, V 2%		
A	0.03	0.035	+0.005
B	0.03	0.028	−0.002
C	0.30	0.270	−0.030
D	0.30	0.274	−0.026

Table III. Alloys with Added Beryllium

Aliquot	Be Added, %	Be Found, %	Difference, %
Alloy 155AX.	Ti 88%, Fe 0.9 to 1.7%, Cr 0.8 to 2.0%, Al 4.0 to 6.0%, Mo 0.8 to 2.0%		
A	0.30	0.29	−0.01
B	0.50	0.41	−0.09
C	1.00	0.99	−0.01
D	1.33	1.34	+0.01
E	2.00	2.08	+0.08
Alloy 140A.	Ti 93.7%, Fe 1.5 to 2.5%, Cr 1.5 to 2.5%, Mo 1.5 to 2.5%		
A	0.50	0.57	+0.07
B	1.00	0.99	−0.01
C	1.50	1.60	+0.10
D	2.00	2.00	0.00
	Alloy 6A1-4V. Ti 90%, Al 6.0%, V 4.0%		
A	0.25	0.30	+0.05
B	0.50	0.50	0.00
C	0.75	0.72	−0.03
D	1.00	1.06	+0.06
E	1.33	1.34	+0.01

In addition ten determinations were made on each of three experimental alloys having beryllium contents of approximately 0.1, 0.25, and 0.50% (Table IV). The standard deviation, in each group of determinations, was less than 0.006%.

The reference solution in each case was a reagent blank containing all reagents used but no sample.

The elements usually present in titanium alloys do not interfere when present in the amounts normally used in commercial products. No interference was observed in the presence of up to 2.5% iron, 2.5% chromium, 6% aluminum, 2.5% molybdenum, or 4% vanadium.

The method was also checked for interference by magnesium; no interference was observed with concentrations of magnesium ranging up to 5% of the sample weight. Hydrofluoric and fluoboric acids interfere when present in the solution. The color does not develop at all in the presence of hydrofluoric acid.

Table IV. Beryllium in Experimental Alloys

Aliquot	X_1, Be, %	$X_1 - X$, Deviation, %
Al 6%, V 4%, Be 0.1%, Ti 90%		
A	0.125	+0.006
B	0.119	0.000
C	0.111	−0.008
D	0.125	+0.006
E	0.115	−0.004
F	0.122	+0.003
G	0.126	+0.007
H	0.116	−0.003
I	0.113	−0.006
J	0.122	+0.003
Ave. $\bar{X}$	0.119	
Standard deviation	±0.005%	
Al 6%, V 4%, Be, 0.25%, Ti 90%		
A	0.244	−0.009
B	0.246	−0.007
C	0.248	−0.005
D	0.256	+0.003
E	0.250	−0.003
F	0.254	+0.001
G	0.256	+0.003
H	0.257	+0.004
I	0.258	+0.005
J	0.256	+0.003
Ave. $\bar{X}$	0.253	
Standard deviation	±0.005%	
Al 6%, V 4%, Be 0.50%, Ti 90%		
A	0.475	+0.007
B	0.472	+0.004
C	0.464	−0.004
D	0.473	+0.005
E	0.464	−0.004
F	0.465	−0.003
G	0.461	−0.007
H	0.472	+0.004
Ave. $\bar{X}$	0.468	
Standard deviation	±0.005%	

CONCLUSION

This method is satisfactory for practical application to the analysis of titanium alloys. The accuracy and precision compare favorably with other colorimetric methods applied in the presence of titanium.

ACKNOWLEDGMENT

The authors wish to acknowledge the helpful supervision of Robert L. Powell, Process Research Supervisor, Titanium Metals Corp. of America.

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RECEIVED for review December 5, 1955. Accepted July 19, 1956. Division of Analytical Chemistry, Symposium on Analysis of Titanium and Its Alloys, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

Sodium Gluconate as a Complexing Agent in the Volumetric Analysis of Aluminum Compounds

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Tartrate has often been used as a complexing agent for aluminum. Several methods have employed barium in conjunction with tartrate to obtain a better end point for the titration of aluminum. In the present work, limited tests revealed certain basic knowledge concerning effects of the structure of complexing agents. It was found that sodium gluconate could satisfactorily replace both tartrate and barium in aluminum titrations. The advantages of gluconate are the lack of sulfate or carbonate interference and the simplicity of a single complexing reagent. Another original feature of the method is the titration of carbonate to bicarbonate in the presence of aluminum. Accurate and precise methods are included for the determination of aluminum oxide or aluminum, acids, hydroxide, and carbonate. The effects of impurities are discussed.

MOST volumetric methods for the determination of aluminum are based upon the following reaction:

$$Al(OH)_3 + 6KF \rightarrow K_3AlF_6 \downarrow + 3KOH \qquad (1)$$

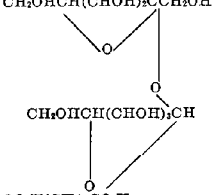
The quantity of aluminum is calculated from the titration of the released hydroxide.

Several investigators have used tartrate salts or tartaric acid to complex the aluminum hydroxide so that it would be soluble during the initial pH adjustment just before the addition of fluoride. Vieböck and Brecher (*5*) formed a barium-aluminum-tartrate complex, neutralized the solution, added fluoride, and titrated the released hydroxide. Snyder (*4*) proposed a variation in which the acidic solution containing the aluminum tartrate complex was neutralized with barium hydroxide. Hale (*2*) substituted lithium for barium, so that the titration could be done in the presence of sulfate. For analysis of aluminate solutions, such as Bayer liquors, the authors (*6*) combined a titration of total hydroxide to the phenolphthalein end point with a stoichiometric modification of Vieböck and Brecher's method for aluminum.

Vieböck and Brecher, Snyder, and Bushey (*1*) recognized that the presence of barium was necessary for accurate pH adjustment of the sample solution before the addition of fluoride. The authors found that a barium-to-aluminum mole ratio of 2 to 1 was required and that strontium could be substituted for barium. Curves for the hydroxide titration in the presence of 0.125 gram of aluminum oxide, shown in Figure 1, demonstrate the effect of tartrate alone, tartrate and lithium, and tartrate and barium.

Lithium did not significantly change the slope of the curve over that with tartrate alone, but barium caused more than a threefold increase in the sharpness of the inflection point.

Table I. Reactions of Various Organic Reagents with Aluminum

Compound	Formula	Reagent Caused Increase in pH	Aluminum Soluble in Neutral Solution
Sodium citrate	$CO_2NaCH_2C(CO_2Na)OHCH_2CO_2Na$	Yes	Yes
Sodium tartrate	$CO_2Na(CHOH)_2CO_2Na$	Yes	Yes
Sodium gluconate	$CH_2OH(CHOH)_4CO_2Na$	Yes	Yes
Mannitol	$CH_2OH(CHOH)_4CH_2OH$	No	Yes
Glycerol	$CH_2OHCHOHCH_2OH$	No	No
Sucrose	$CH_2OHCH(CHOH)_2CCH_2OH$ … $CH_2OHCH(CHOH)_3CH$ 	No	No
Potassium succinate	$CO_2K(CH_2)_2CO_2K$	No	No
Sodium 2-ethyl hexoate	$CH_3(CH_2)_3CH(C_2H_5)CO_2Na$	No	No
Sodium propionate	$CH_3CH_2CO_2Na$	No	No
Sodium malonate	$CH_2(CO_2Na)_2$	No	No

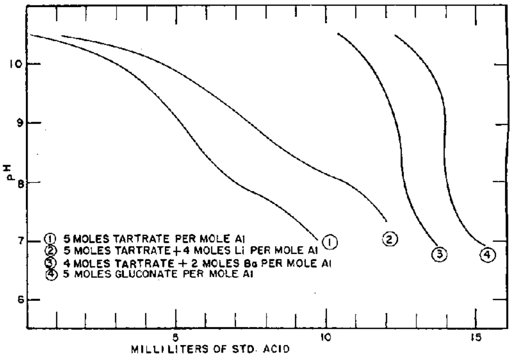


Figure 1. Titration curves for total hydroxide in presence of various reagents

Sulfate and carbonate are precipitated by barium. The filtered precipitate of barium carbonate has been used for carbonate analysis (*1*, *6*), and hydroxide and aluminum can be determined in the filtrate. However, precipitates of barium carbonate and barium sulfate are adsorptive, and high carbonate and low hydroxide values are obtained.

A complexing reagent was needed that would permit sharp end points, be free of sulfate and carbonate interference, and form a fully associated complex with aluminum that can be completely decomplexed by the addition of fluoride, and reactions should be stoichiometric.

INVESTIGATION OF COMPLEXING AGENTS

Ten organic reagents, selected for their structures were individually tested as follows:

A sodium aluminate solution containing 0.2 gram of aluminum oxide was titrated with hydrochloric acid to the first trace of precipitation of aluminum hydroxide. The solution was then titrated with a neutral (to phenolphthalein) 0.4*M* solution of the organic reagent until the pH ceased to rise (with seven of the reagents there was no rise). An increase in pH indicated release of combined hydroxide. In a second test a mixture of the aluminate solution and the organic reagent was neutralized to about pH 8.3 with hydrochloric acid to find whether the aluminum was complexed against precipitation as aluminum hydroxide. In each test, 0.2 gram of aluminum oxide and at least 4 moles of organic reagent per mole of aluminum were used. The results are shown in Table I.

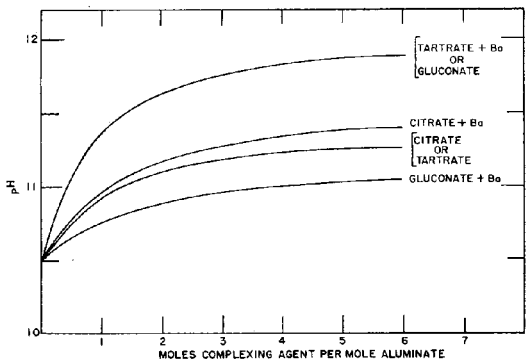


Figure 2. Titration of sodium aluminate with complexing agents

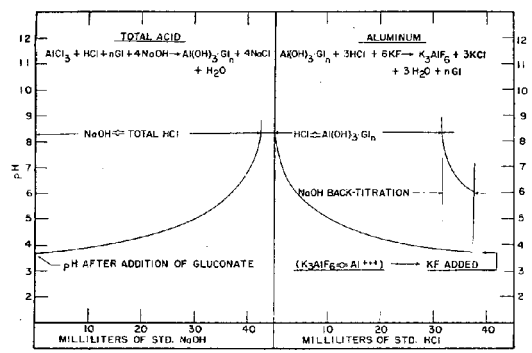


Figure 3. Titration curves for total acid and aluminum

Citrate, tartrate, gluconate, and mannitol complexed aluminum against precipitation. Each of these reagents contains one to six hydroxyl groups on chains consisting of four to six carbon atoms. Sucrose and glycerol, which have hydroxyl groups but longer and shorter chains, respectively, failed in this test. Citrate, tartrate, and gluconate released combined hydroxide. They contain one to three carboxyl groups and one to five hydroxyl groups. Mannitol, which differs from gluconate only in the lack of a carboxyl group, complexed aluminum against precipitation but did not release combined hydroxide.

Figure 2 shows curves of 0.2 gram of aluminum oxide complexed with citrate, tartrate, and gluconate, in both the presence and absence of 2.5 grams of barium chloride dihydrate. The mole ratios of reagent to aluminum were plotted against rise in pH caused by release of combined hydroxide. The curves show that barium increases the effectiveness of tartrate and citrate, which contain two and three carboxyl groups, respectively; but barium impaired the effectiveness of gluconate, which has only one carboxyl group. The curves in Figure 1 show that the inflection in the presence of gluconate alone is as sharp as that in the presence of both tartrate and barium.

Sodium gluconate met the desired qualifications for a complexing agent. Procedures were then developed for complete analysis of both acidic and basic solutions of aluminum.

CHEMISTRY OF THE PROCEDURES

Though sources differ concerning the exact reactions of complexing agents, some simple equations are included here for better understanding of the reactions. In the equations sodium gluconate is represented by Gl. Typical titration curves of the reactions are also shown in Figures 3 and 4.

Total Acid. Free acid and the combined acid released by complexing aluminum with sodium gluconate are titrated to pH 8.3 with standard sodium hydroxide, as shown in Figure 3. The reaction may be represented as follows:

$$AlCl_3 + HCl + n\text{Gl} + 4NaOH \rightarrow Al(OH)_3.\text{Gl}_n + 4NaCl + H_2O \quad (2)$$

Total Soda (Total Hydroxide Plus Total Carbonate). In the first part of the reaction, the sample containing sodium aluminate, sodium carbonate, and free sodium hydroxide is acidified with excess hydrochloric acid. The carbon dioxide is evolved by boiling.

$$NaAlO_2 + Na_2CO_3 + NaOH + 8HCl \rightarrow AlCl_3 + HCl + CO_2\uparrow + 4NaCl + 4H_2O \quad (3)$$

After the sample is cooled to room temperature, sodium gluconate is added. Free acid and the combined acid released by complexing the aluminum are titrated to pH 8.3, as in the total acid determination (Figure 3 and Equation 2). The net hydrochloric acid titration from Equations 3 and 2 represents total soda.

Hydroxide Plus One-Half Carbonate. The familiar titration of carbonate to bicarbonate can be performed in an alkaline solution of aluminum that is complexed with sodium gluconate. However, pH 8.1 is the inflection midpoint, whereas pH 8.3 is usually selected in the absence of aluminum. The correct end point is important because the titration is buffered.

Sodium gluconate is added to the sample containing sodium aluminate, sodium carbonate, and free sodium hydroxide. Then, as shown in Figure 4, the sample is titrated to pH 8.10 with standard hydrochloric acid.

$$NaAlO_2 + Na_2CO_3 + NaOH + n\text{Gl} + 3HCl \rightarrow Al(OH)_3.\text{Gl}_n + NaHCO_3 + 3NaCl \quad (4)$$

Aluminum Oxide or Aluminum. The aluminum determination can be performed after titration of total acid, total soda, or hydroxide-bicarbonate, since, after any of the three titrations, aluminum is present as the gluconate complex and the solution has been titrated to either pH 8.3 or 8.1. Either pH 8.3 or 8.1 can serve as the starting pH of the aluminum titration, provided the same pH is used for the final end point. In the recommended method (Figure 3) standard hydrochloric acid is added at this point in an amount sufficient to give an excess over that required to neutralize the hydroxide present in the $Al(OH)_3.\text{Gl}_n$ complex. Potassium fluoride is then added, the three equivalents of hydroxide per equivalent of aluminum are released, and the remaining excess hydrochloric acid is back-titrated to the starting pH of 8.3. The alternative method (Figure 4) is very similar. The only difference is that the fluoride is added at the

start of the aluminum titration instead of after the addition of excess hydrochloric acid. The hydroxide released by the addition of fluoride is titrated with hydrochloric acid, excess hydrochloric acid is added, and the excess hydrochloric acid is back-titrated with standard sodium hydroxide.

PROCEDURES

Apparatus. pH meter. Magnetic or mechanical stirrer.

Reagents. Sodium gluconate (Pfizer, refined), 20% solution. Store in polyethylene. Adjust to phenolphthalein neutrality.

Standard hydrochloric acid and sodium hydroxide solutions, 0.3 to 0.4*N*.

Potassium fluoride solution, 50% $KF.2H_2O$ by weight, filtered, and stored in polyethylene. Adjust the solution with sodium hydroxide and hydrochloric acid until 25 ml. of the potassium fluoride solution, when added to about 250 ml. of water and 50 ml. of sodium gluconate solution at about pH 8.3 or the phenolphthalein end point, will change the pH by not more than the equivalent of 1 drop of standard hydrochloric acid or sodium hydroxide.

Phenolphthalein, 0.1% solution.

Distilled water. The distilled water should be boiled and cooled for greatest accuracy in titrations of hydroxide, carbonate, or total acid.

Procedure. INITIAL PREPARATION OF ALL SAMPLES. Pipet an aliquot of sample containing from 0.025 to 0.25 gram of aluminum oxide (0.013 to 0.13 gram of aluminum) into a 600-ml. beaker. Add several drops of phenolphthalein indicator. If the sample is colorless to phenolphthalein, use Procedures I and V. If pink to phenolphthalein, use Procedures II and V on one aliquot and Procedures III and V on a duplicate aliquot.

I. TITRATION OF TOTAL ACID. Add 50 ml. of sodium gluconate solution to the sample, and dilute it to about 250 ml. Titrate to pH 8.3 with standard sodium hydroxide.

Grams of HCl = ml. of NaOH × normality × 0.03646
Grams of H_2SO_4 = ml. of NaOH × normality × 0.04904

II. TITRATION OF TOTAL SODA (Total Hydroxide and Total Carbonate). Add standard hydrochloric acid (a recorded amount) to the sample until aluminum hydroxide precipitates and is redissolved by stirring. Cover the beaker with a watch glass. Bring the sample to a boil, and allow it to simmer for about 10 minutes. Cool the sample to room temperature, wash down the watch glass and sides of the beaker, add 50 ml. of sodium gluconate solution, and dilute the sample to about 250 ml. Titrate to pH 8.3 with standard sodium hydroxide.

Grams of NaOH (as Na_2CO_3) plus grams of Na_2CO_3 =
(ml. of HCl − ml. of NaOH) × normality × 0.053

III. TITRATION OF TOTAL HYDROXIDE PLUS ONE-HALF CARBONATE. Add 50 ml. of sodium gluconate solution to the sample, and dilute it to about 250 ml. Titrate with standard hydrochloric acid to pH 8.10.

Grams of NaOH (as Na_2CO_3) plus $^1/_2$ grams of Na_2CO_3 =
ml. of HCl × normality × 0.053

IV. HYDROXIDE AND CARBONATE CALCULATIONS.

Grams of Na_2CO_3 = 2 × (result from II − result from III)
Grams of NaOH (as Na_2CO_3) = result from II − grams of Na_2CO_3

V. TITRATION OF ALUMINUM OXIDE OR ALUMINUM. Continue the titration of the sample from either I or II by either the recommended or alternative method. For titration of the sample from III, use only the alternative method.

A. Recommended Method. Add 25 ml. of standard 0.3*N* hydrochloric acid if the sample contains less than 0.105 gram of aluminum oxide or 50.0 ml. of hydrochloric acid if the sample contains from 0.105 to 0.21 gram of aluminum oxide. When using 0.4*N* acid and base, adjust the amounts proportionately. If the approximate content of aluminum oxide is known, add enough hydrochloric acid to provide a 5- to 10-ml. excess after release and neutralization of the combined hydroxide. Add 25 ml. of potassium fluoride solution. Back-titrate with standard sodium hydroxide to pH 8.3.

B. Alternative Method. Add 25 ml. of potassium fluoride solution. Titrate with standard hydrochloric acid at the full speed of the buret until the sample is colorless to phenolphthalein; then add a 5- to 10-ml. excess of acid. Back-titrate with standard sodium hydroxide to the starting pH (8.3 or 8.1).

Grams of Al_2O_3 =
(ml. of HCl − ml. of NaOH) × normality × 0.01699

Grams of Al =
(ml. of HCl − ml. of NaOH) × normality × 0.00899

DISCUSSION OF PROCEDURES

Phenolphthalein end points can be used for all titrations except the hydroxide-bicarbonate (III) titration and the aluminum titration in the presence of carbonate.

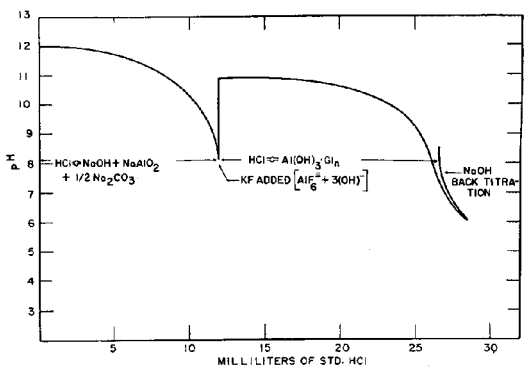


Figure 4. Titration curves for total sodium hydroxide plus one-half sodium carbonate and aluminum

In all titrations the stirring rate should be slower than that at which air bubbles are whipped into the solution. The slow stirring rate helps to avoid pickup of atmospheric carbon dioxide.

If the aluminum oxide (aluminum) titration is the only one desired, the initial adjustment to approximately pH 8.3 can be simplified by the use of more concentrated solutions of sodium hydroxide and hydrochloric acid.

Combined acid or combined sodium hydroxide can be calculated from the aluminum result. A value for free acid or free sodium hydroxide can be obtained by subtracting from the total acid or total sodium hydroxide the combined acid or combined sodium hydroxide.

In the procedures separate sample aliquots are used for the hydroxide-bicarbonate and the total soda titrations; however, aluminum can be determined on either or both samples. Actually, all three titrations can be performed on a single sample aliquot, as follows:

Perform the hydroxide-bicarbonate titration, add excess standard acid, boil out the carbon dioxide, neutralize with sodium hydroxide and add a small excess, boil again, cool, complete the total soda determination by titrating to pH 8.3 with hydrochloric acid, and titrate the aluminum.

In using this variation, two points must be emphasized. The addition of excess hydrochloric acid and boiling convert sodium gluconate to a rather stable γ-lactone form of gluconic acid (*7*). The reconversion to sodium gluconate, without which inaccurate values are obtained for total soda, requires boiling the sample in the presence of excess sodium hydroxide (*8*). Also, because the lactone conversion of the gluconic acid can result in partial dissociation of the aluminum complex, enough acid should be present to convert all aluminum to aluminum chloride.

Table II. Determination of Total Hydrochloric Acid and Aluminum Oxide in Hydrochloric Acid–Aluminum Chloride Solution

	Total HCl		
Added, g.		Av. bias, mg.	Range of duplicates, mg.
0.1062		+0.2	0.0
0.2124		+0.3	0.0
0.4249		−0.2	0.3
0.5311		+0.9	0.2
0.7435		+0.9	0.1
0.8498		+0.7	0.1
Av. 0.4780		+0.5	0.12
		Std. dev.[a]	0.11

	Al_2O_3			
	Recommended Method		Alternative Method	
Added, g.	Av. bias, mg.	Range of duplicates, mg.	Av. bias, mg.	Range of duplicates, mg.
0.0251	+0.4	0.0	+0.4	0.2
0.0501	+0.5	0.2	+0.4	0.2
0.1003	−0.2	0.1	+0.2	0.1
0.1253	+0.3	0.1	−0.3	0.1
0.1754	+0.3	0.1	−0.2	0.1
0.2005	−0.5	0.4	−0.9	0.4
Av. 0.1128	+0.1	0.15	−0.1	0.18
	Std. dev.[a]	0.14		0.15

[a] Std. dev. $(s) = \sqrt{\frac{\Sigma d^2}{2n}}$, where d is difference between duplicates and n is number of duplicate pairs.

Table III. Determination of Total Sulfuric Acid and Aluminum Oxide in Sulfuric Acid–Aluminum Sulfate Solution

	Total H_2SO_4		
Added, g.		Av. bias, mg.	Range of duplicates, mg.
0.2455		+1.0	0.1
0.4909		+0.5	0.4
0.9819		+0.4	0.4
1.2273		−0.2	0.9
1.7182		+1.0	1.2
1.9637		+1.4	0.8
Av. 1.1046		+0.7	0.63
		Std. dev.[a]	0.52

	Al_2O_3			
	Recommended Method		Alternative Method	
Added, g.	Av. Bias, mg.	Range of duplicates, mg.	Av. bias, mg.	Range of duplicates, mg.
0.0255	+0.9	0.1	+0.4	0.2
0.0510	+0.7	0.2		
0.1021	+0.1	0.3	−0.4	0.1
0.1276	+0.8	0.0		
0.1786	+0.7	0.1		
0.2042	+0.0	0.1	−1.5	0.4
Av. 0.1148	+0.5	0.13		
	Std. dev.[a]	0.11		

[a] Std. dev. $(s) = \sqrt{\frac{\Sigma d^2}{2n}}$, where d is difference between duplicates and n is number of duplicate pairs.

PREPARATION OF SYNTHETIC SOLUTIONS

Solutions of hydrochloric acid, sulfuric acid, and a mixture of sodium hydroxide and sodium carbonate were prepared and carefully analyzed. Then, weighed amounts of 99.988% pure aluminum wire were dissolved in aliquots of the solutions.

In the case of the acids, the aluminum solutions were prepared in a flask with a ground joint attached to a reflux condenser. For the sodium hydroxide–sodium carbonate solution, a larger flask was used, and it was connected to a trap which was vented through an Ascarite tube to prevent absorption of carbon dioxide.

Heat was applied in each case to hasten the reaction. The aluminum dissolved in a few hours in solutions of either hydrochloric acid or sodium hydroxide–sodium carbonate. When sulfuric acid was used, however, complete solution of the aluminum required several days, even though mercuric chloride (1.2 mg. per gram of aluminum) was used as a catalyst.

After the aluminum had dissolved completely, the solutions were diluted to volume at room temperature in volumetric flasks. After thorough mixing, each solution was transferred to a polyethylene bottle.

PRECISION AND ACCURACY

Recoveries and standard deviations are shown in Tables II, III, and IV.

Table IV. Determination of Aluminum Oxide, Sodium Hydroxide, and Sodium Carbonate in Sodium Aluminate Solutions

	Na_2CO_3, G.		NaOH (as Na_2CO_3), G.		Al_2O_3 (by Recommended Method), G.	
	Added	Found	Added	Found	Added	Found
			Solution I			
	0.2822	0.2850	0.5431	0.5416	0.1269	0.1270
		0.2848		0.5415		0.1271
		0.2850		0.5416		0.1269
		0.2844		0.5424		0.1270
		0.2844		0.5419		0.1271
		0.2854		0.5412		0.1269
Av. found, g.		0.2848		0.5417		0.1270
Av. bias, mg.		+2.6		−1.4		+0.1
Std. dev.,[a] mg.		0.39		0.41		0.09
			Solution II			
	0.2822	0.2856	0.5431	0.5413	0.2505	0.2500
		0.2864		0.5405		0.2500
		0.2846		0.5418		0.2501
		0.2822		0.5432		0.2498
		0.2848		0.5419		0.2499
		0.2838		0.5424		0.2498
Av. found, g.		0.2846		0.5419		0.2499
Av. bias, mg.		+2.4		−1.2		−0.6
Std. dev.,[a] mg.		1.46		0.92		0.13

[a] Std. dev. $(s) = \sqrt{\frac{\Sigma(X - \bar{X})^2}{N - 1}}$, where $(X - \bar{X})$ is deviation of each determination from average and N is number of determinations.

Table V. Effect of Impurities on Aluminum Oxide Results

Actual Reagent Tested	Equivalent Oxide	Approximate Amount (G.) of Oxide Impurity That Produced Significant Bias	Sign of Bias Produced
$Na_2SiO_3.5H_2O$	SiO_2	<0.001	+
$BeSO_4.4H_2O$	BeO	<0.001	+
$FeSO_4.7H_2O$	FeO	<0.001	+
$FeCl_3.6H_2O$	Fe_2O_3	<0.001	+
$MnCl_2.4H_2O$	MnO	<0.001	+
$Zr(NO_3)_4$	ZrO_2	0.001	+
$Ti(SO_4)_2$	TiO_2	0.002	+
$Ga_2(SO_4)_3$	Ga_2O_3	0.002	+
$Th(NO_3)_4.4H_2O$	ThO_2	0.002	+
$SnCl_2.2H_2O$	SnO	0.002	+
$NaVO_3.16H_2O$	V_2O_5	0.002	+
$ZnSO_4.7H_2O$	ZnO	0.003	+
$UO_2(NO_3)_2.6H_2O$	UO_3	0.004	+
$NiCl_2.2H_2O$	NiO	0.005	+
$CoSO_4.7H_2O$	CoO	0.008	+
$Ce(NO_3)_3.6H_2O$	Ce_2O_3	0.01	+
$BaCl_2.2H_2O$	BaO	0.01	−
$CrCl_3.6H_2O$	Cr_2O_3	0.015	+
$Na_2C_2O_4$	...	0.02	−
NaF	...	0.025	−
$Na_2HAsO_4.7H_2O$	As_2O_5	0.025	−
$CuCl_2$	CuO	0.03	+
$CdCl_2.2^1/_2H_2O$	CdO	0.03	+
$Na_2WO_4.2H_2O$	WO_3	0.03	+
$Na_2PO_4.12H_2O$	P_2O_5	0.04	−
H_3BO_3	B_2O_3	0.06	−
$NaAsO_2$	As_2O_3	0.10	−
$CaCl_2$	CaO	0.12	+
$Na_2CrO_4.4H_2O$	CrO_3	0.13	−
$MgSO_4.7H_2O$	MgO	0.14	+
$Na_2MoO_4.2H_2O$	MoO_3	>0.2	
$SrCl_2$	SrO	>0.2	
LiCl	Li_2O	>0.2	

Results for total hydrochloric acid in Table II averaged 0.5 mg. high. This is equivalent to 0.05 ml. of 0.3*N* hydrochloric acid. The standard deviation was 0.1 mg. This was good accuracy and excellent precision. Results for total sulfuric acid in Table III averaged 0.7 mg. or 0.05 ml. high, and the standard deviation was 0.5 mg. The accuracy was good, and precision was acceptable.

The sodium carbonate results shown in Table IV averaged 2.5 mg. high and had standard deviations of 0.4 and 1.5 mg. The sodium hydroxide results averaged 1.3 mg. low, and the standard deviations were 0.4 and 0.9 mg. The greatest error was in carbonate, which averaged about 0.9% high, corresponding to 0.12 ml. of 0.4*N* hydrochloric acid. As can be seen from the recommended procedures, error in the total soda or hydroxide-bicarbonate titrations will be doubled in the carbonate and hydroxide values. However, these results were considerably better than those from former procedures (*1*, *6*), in which the carbonate is precipitated as barium carbonate, filtered off, and analyzed. In such methods significant errors are caused by adsorption by barium carbonate and the reaction of sodium hydroxide with atmospheric carbon dioxide during filtration. Therefore, though the accuracy and precision for carbonate and hydroxide were not as good as was desired, the results were an improvement over those by previous procedures.

Average biases for aluminum oxide by the recommended method were +0.1, +0.5, +0.1, and −0.6 mg. (Tables II, III, and IV). The standard deviation estimates were all about 0.1 mg. This is good accuracy and excellent precision. The recommended method was unaffected by aluminum oxide concentration. In contrast, the alternative method (Table II) gave a +0.4-mg. bias at the 0.025 gram of aluminum oxide concentration, and the biases progressed to −0.9 mg. at the 0.2 gram of aluminum oxide concentration. However, results in the 0.05 to 0.15 gram of aluminum oxide concentration range were satisfactory. The alternative method requires less standard hydrochloric acid and sodium hydroxide, and it is better adapted to samples of completely unknown aluminum oxide concentration.

Results for aluminum oxide in the presence of nitric and perchloric acids had accuracy and precision corresponding to that shown in Tables II and III.

EFFECT OF IMPURITIES ON ALUMINUM OXIDE RESULTS

Aliquots of several aluminum chloride solutions containing about 0.2 gram of aluminum oxide were titrated by the recommended method. Samples containing no impurity were interspersed at random among samples to which a solution containing a known amount of impurity had been added.

A pooled value for standard deviation was obtained for samples to which no impurity had been added. Control limits were arbitrarily established as the average without impurity ± 3 times the pooled standard deviation estimate ($\bar{X} \pm 3s$).

Each impurity was tested to find the amount, if less than 0.2 gram as the anhydrous oxide, that would cause an aluminum oxide bias equal to the control limit. Titration curves were prepared on the impurities in amounts equivalent to 0.2 gram of anhydrous oxide. These tests were limited, and the data are recommended only as a guide. The results of the tests are shown in Table V.

The 16 impurities that produced the strongest interferences had positive biases in aluminum oxide. The titration curves showed that the greatest cause of interference was the release of hydroxide under the conditions of the method. Intermediate interference was caused by a combination of hydroxide release and buffering, and buffering was the principal cause of minor interference.

CONCLUSION

The methods presented provide for accurate, precise, and rapid determination of aluminum oxide or aluminum, total acid, hydroxide, and carbonate.

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RECEIVED for review March 22, 1956. Accepted July 30, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 28, 1956.

Direct Counting of Tritium-Tagged Solid and Liquid Samples

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The direct counting of tritium-tagged solid and liquid samples has been found to be reproducible with a standard deviation of 4 and 6% for infinitely thick and infinitely thin samples, respectively.

THIS study was undertaken to investigate the direct counting of tritium-tagged triglycerides, fatty acids, and soaps. The counting behavior of both infinitely thin and infinitely thick samples of these materials was studied over 20 to 37 days in order to determine their stability during practical counting periods.

The usual methods of counting the radioactivity of tritium-tagged samples involve converting hydrogen of the sample into gaseous hydrogen (*1*, *7*, *10*) or a hydrocarbon (*3*, *8*, *9*), filling a gas tube, and counting the activity with a Geiger, proportional, or vibrating reed electrometer system. These methods are accurate, in general, and offer high counting efficiency. Jenkins (*5*) described a novel method of counting the radioactivity of tritiated water, by exchanging its activity with ammonium chloride followed by subliming and counting of the ammonium chloride sample. Recently Hayes and Gould (*4*) counted tritium radioactivity by dissolving tritium-containing materials, such as steroids and fatty acids, in liquid scintillation systems. They obtained counting efficiencies of 40 to 60%.

The work of Eidinoff and Knoll (*2*), who reported on the direct counting of solid samples of tritium-tagged methyl-3-α-acetoxychlorinate, is more closely related to the present work than that of the previously cited investigators.

The present work confirms that of Eidinoff and Knoll and extends it to include the counting of liquid as well as solid samples. In addition, data are presented to show that tritium activity

may be satisfactorily counted as infinitely thin (standard deviation 6%) or liquid infinitely thick (standard deviation 4%) samples. Further, results are given to show that both infinitely thick and infinitely thin samples give reproducible, constant counts over a 20- to 37-day period.

Direct counting methods have the obvious advantages over the gas conversion methods of simplicity, speed, and nondestruction of samples.

EXPERIMENTAL

Materials. Tritium-tagged cottonseed oil triglycerides were prepared by partial hydrogenation of the oil with a mixture of hydrogen and tritium. Specifically, 20 grams of cottonseed oil were mixed with 20 grams of ethyl acetate and 0.05% of palladium-charcoal catalyst (as 5% palladium on charcoal) in a Parr hydrogenation bottle. To this there was added a sealed vial containing 200 mc. of tritium. The Parr setup was assembled and put under 50 pounds per square inch of hydrogen pressure. The bottle was shaken (breaking the tritium vial) for 24 minutes. The iodine value of the cottonseed oil dropped from 110 to 40 during the tritiumation.

Cottonseed fatty acids-H^3 were prepared by the usual saponification acidification procedure.

Cottonseed fatty acid soaps-H^3 were prepared by titration of an alcohol solution of the fatty acids with alcoholic sodium hydroxide.

Counting Sample Preparation. In general, two types of counting samples were prepared. Samples of infinite thickness (>0.9 mg. of material per sq. cm.) were prepared by weighing 100 mg. of material into a cupped nickel-iron planchet 1 inch in diameter and 5/16 inch deep. A piece of lens paper 1 inch in diameter was then added to each sample to keep it in a uniformly spread condition.

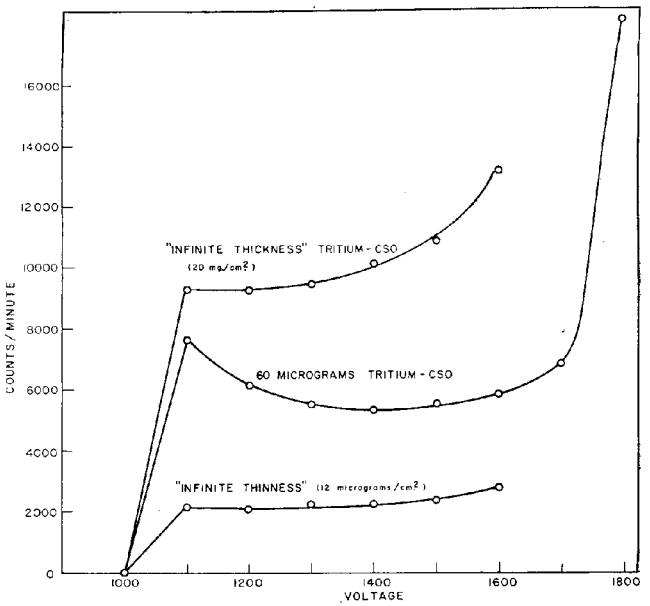

Figure 1. Operational characteristics of windowless gas-flow counter

The "infinitely thin" samples (<23 γ per sq. cm.) were prepared by pipetting 1 ml. of a solvent containing the sample into the cupped planchet, followed by evaporation of the solvent.

Radio Counting Equipment and Procedure. A windowless gas-flow "modified" Geiger counter (Research Equipment Co.) was used for radioassay of the tritium-tagged samples. This counter, a loop anode type, operates at 1200 volts using 99% helium–1% isobutane gas. It has a coincidence loss of only 2% at 50,000 counts per minute, when a preamplifier is used between it and the scaler.

For reproducibility of counting, it was found necessary to keep all infinitely thick samples melted during counting. This was accomplished by heating the counter to 45° to 50° C. with a heating mantle placed under the counter.

Counter Operational Characteristics. The operational characteristics of the gas-flow counter are shown in Figure 1. The counting plateaus of the tritium samples are satisfactory for good counting operation. No explanation is known for the dip in the counting plateau of the 60-γ tritium-tagged cottonseed oil (CSO) sample.

RESULTS

Self-Absorption for Tritium-Tagged Samples. The self-absorption characteristics of tritium-tagged cottonseed oil samples are given in Figure 2. The curves in Figure 2 are typical of those obtained for tritium-tagged cottonseed fatty acid samples and cottonseed soap samples. These curves indicate that self-absorption is negligible up to sample weights of about 23 γ per sq. cm. Above this sample weight, self-absorption becomes increasingly important. Accordingly, in this work, infinitely thin samples were considered to be those of <23 γ per sq. cm. and infinitely thick samples those of >0.9 mg. per sq. cm. [maximum range of tritium radiation is 0.82 mg. per sq. cm. as calculated by means of Libby's (*6*) range equation].

Stability of Radio Counting Data with Time for Tritium-Tagged Samples. Periodic counting of tritium-tagged cottonseed oil, fatty acid, and soap samples at 2- to 4-day intervals over a 20- to 37-day period showed no change in radioactivity. The infinitely thick samples must be kept in a liquid state during counting, in

Table I. Comparison of Counts of Liquid and Solid Tritium-Tagged Cottonseed Oil Triglyceride Samples[a]

Operator	Sample Counts 1	2	3	Mean	Standard Deviation, %
		Liquid Samples			
A	13,821	13,544	12,548	13,304	5.7
B	14,777	14,455	15,167	14,800	2.9
C	14,438	13,397	14,017	14,284	1.8
			Av.	14,129	
		Standard deviation = 6.2%			
		Solid Samples			
A	23,527	19,508	23,274	22,103	10.8
B	29,048	30,437	33,336	31,240	6.4
C	38,142	44,889	39,413	40,815	9.8
			Av.	31,386	
		Standard deviation = 35.0%			

[a] 100-mg. samples, lens paper, 1 1/8 × 1/16 inch Al planchets.

Table II. Standard Deviation of Counts of Liquid Samples

(100 ± 5 mg. samples)

Source of Variability	Standard Deviation, % 1 × 5/16 inch planchet	1 1/8 × 1/16 inch planchet
With Lens Paper (15/16 inch Diameter; 1.1 Mg. per Sq. Cm.)		
Replicate samples, same operator	3.5	4.4
Replicate samples, 3 different operators	3.6	6.5
Different operators (3)	2.6	5.1
Date counted (same sample counted on 7 days)	3.8	4.6
Over-all precision	4	6
Without Lens Paper		
Replicate samples, same operator	18	13

order to obtain acceptable accuracy and reproducibility. This fact is brought out clearly by the data given in Table I, in which the counts of liquid samples are compared with those of solid samples. Although the parent material used is identical in Table I, the solid samples have an average count over twice as great as that of the liquid phase. This is thought to be a result of the much greater surface area of the crystalline solid samples.

Stability of count data with time was not taken for an infinitely thick cottonseed oil soap sample, because soaps do not melt at practical counting temperatures, and it is considered to be of little value to count a solid infinitely thick sample in view of the very large variability in the counts of this type of sample. Drifting of the count rate was sometimes observed for infinitely thick solid, but not for liquid-type samples.

Standard Deviation of Counts of Liquid and Infinitely Thin Tritium-Tagged Samples. The standard deviations of several variables involved in preparing and counting tritium-tagged liquid samples are summarized in Table II. All the variables are roughly the same in magnitude, when lens paper is used to spread the samples, averaging about 4% standard deviation for samples counted in the standard 1 × 5/16 inch planchets. The data of Table II bring out two other important points—that elimination of the use of lens paper to spread the liquid samples increases the standard deviation from 3.5 to 18% and the use of 1 1/8 × 1/16 inch aluminum planchets (with lens paper) leads to a higher standard deviation than that found with the 1 × 5/16 inch nickel-iron planchets.

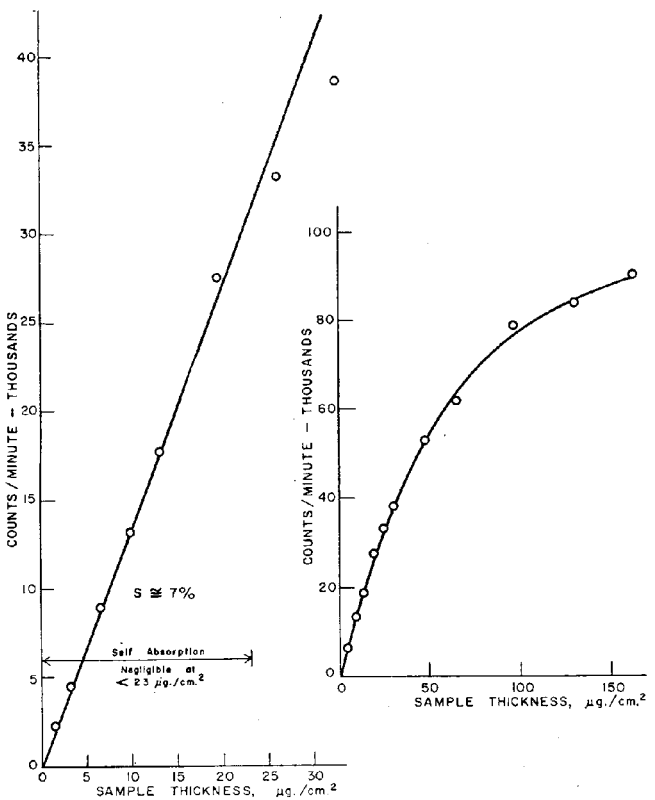

Figure 2. Self-absorption of tritium-tagged cottonseed oil triglyceride samples

1 1/8 × 1/16 inch aluminum planchets
Solution volume constant at 1 ml.

Table III. Standard Deviation of Counts of Infinitely Thin Tritium Samples[a]

(12 γ per sq. cm. sample thickness)

Operator	Type of Material: Fatty Acid, Sample Counts 1	Fatty Acid 2	Soap 1	Soap 2
A	9000	9800	10,580	9,990
B	9280	9490	10,710	9,850
C	9170	9730	10,090	10,670
Mean	9410		10,315	
	Standard Deviation, %			
Between duplicates, same operator	5		6	
Between operators	2		4	

[a] 1.0 ml. of sample solution evaporated in 1 × 5/16 inch planchets.

The over-all precision of infinitely thin tritium-tagged cottonseed oil, fatty acid, and soap samples is about 6% standard deviation. Representative data are given in Table III. Infinitely thin samples can be satisfactorily counted at temperatures below their melting point.

DISCUSSION AND CONCLUSIONS

The direct counting radioassay of tritium-tagged cottonseed oil triglyceride and fatty acid as infinitely thick or infinitely thin samples is satisfactory, using a windowless gas-flow counter. Cottonseed oil soap samples may be counted only as infinitely thin samples, in view of the large standard deviation of the counts of solid infinitely thick samples.

Based on this work it would seem possible to count accurately any nonvolatile tritium-tagged samples as infinitely thin samples or liquid infinitely thick samples.

The precision of sample preparation and of counting shows about 4% standard deviation for liquid 100-mg. samples spread with lens paper and about 6% for infinitely thin samples.

The counting efficiency for infinitely thin tritium-tagged samples counted in the windowless gas-flow counter is 50 ± 5%. Based on this figure, the counting efficiency for 20 mg. per sq. cm. tritium-tagged samples is within about 0.2%. Decreasing the infinitely thick sample size would increase counting efficiency.

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RECEIVED for review April 9, 1956. Accepted July 13, 1956.

Determination of Traces of Selenium
3,3′-Diaminobenzidine as Selenium(IV) Organic Reagent

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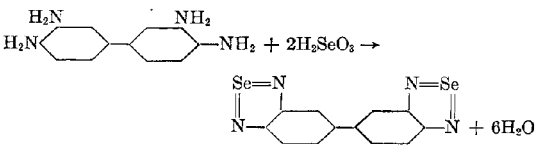
A spectrophotometric study of the selenium(IV) 3,3′-diaminobenzidine reaction was made for the determination of traces of selenium. The yellow selenium compound developed at pH 2 to 3, diphenylpiazselenol, is partially extracted by toluene at pH below 3 but quantitatively extracted at pH above 5. The extraction procedure makes the method more selective, because the colored salt solution does not interfere. Most common ions do not react with diaminobenzidine. The reaction can be carried out in the presence of ethylenediaminetetraacetic acid, which may be used for masking the polyvalent metals. Selenium is determined satisfactorily in the presence of iron, copper, molybdate, titanium, chromium(III), nickel, cobalt, tellurium, and arsenic, and up to 5 mg. of vanadium(V). The strong oxidizing and reducing agents must be absent. The limit of sensitivity of the method is 50 p.p.b. with a 1-cm. absorption cell.

SELENIUM is generally determined by reduction to the reddish elemental selenium with reducing agents such as sulfur dioxide, sulfite, and ascorbic acid. The reduction methods are not only insensitive but also subject to interference from other elements. Přibil (*5*) and Cheng (*1*) reported a new reduction method for selenium with ferrous ethylenediaminetetraacetic acid [(ethylenedinitrilo)tetraacetic acid]. This method is more selective but is still not very sensitive.

Pien (*4*) described an interesting reagent, 3,3′-diaminobenzidine, for determining diacetyl in butter. Later Hoste (*2*) and Hoste and Gillis (*3*) reported a sensitive method for determining selenium by the same reagent. 3,3′-Diaminobenzidine reacts with selenium, forming an intense yellow compound, piazselenol:



According to Hoste (*2*), this reaction was found to be specific, for selenium, after a large number of elements were tested. He reported that oxidizing agents interfered and that iron(III), copper(II), and vanadium reacted with the reagent. The interference from iron(III) can be eliminated by addition of fluoride or phosphoric acid, and interference from copper by oxalic acid.

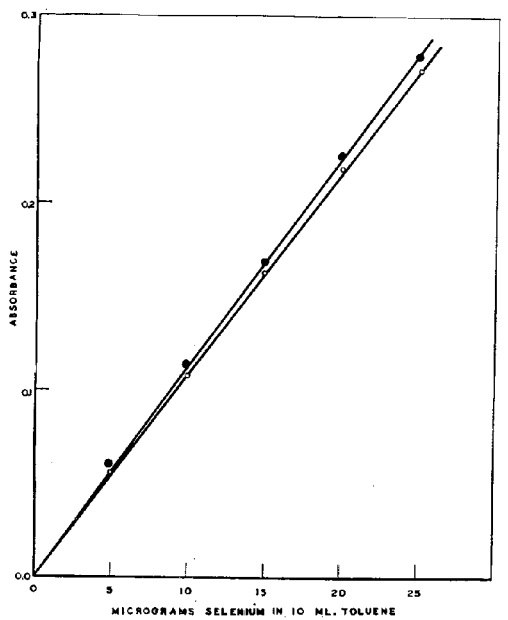


Figure 1. Calibration curves

● 340 mμ
○ 420 mμ

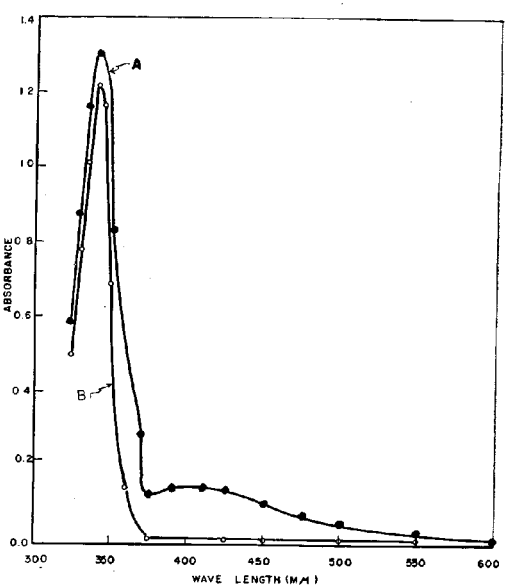


Figure 2. Absorbance curves of aqueous solution

A. 25 γ of Se in 25 ml. of water
B. Diaminobenzidine in water
Water as blank

Table I. Reaction of Diaminobenzidine with Foreign Ions in Presence of EDTA and Tartaric Acid

Ion[a]	Aqueous Solution		Toluene Extraction at pH 6
	Acid	Alkaline	
Silver(I)	White precipitate	Colorless	Colorless
Mercury(II)	Yellow	Yellow precipitate	Colorless
Copper(II)	Green	Black	Colorless
Gold(III)	Brown precipitate	Brown precipitate	Colorless
Tellurium(IV)[b]	Yellow	Yellow	Yellow
Molybdenum(VI)	Colorless	Colorless	Colorless
Tungsten(VI)	Colorless	Colorless	Colorless
Vanadium(V)	Yellowish brown	Yellow	Colorless
Iron(III)	Yellow	Yellow	Colorless
Chromium(III)	Light green	Light green precipitate	Colorless
Uranium(VI)	Yellow	Yellow	Colorless
Titanium(IV)	Light yellow	Colorless	Colorless
Cobalt(II)	Pink	Pink	Colorless
Nickel(II)	Green	Green	Colorless

[a] 1 mg. per ml. solution used.
[b] Reagent grade tellurium dioxide always contains small amounts of selenium (*8*).

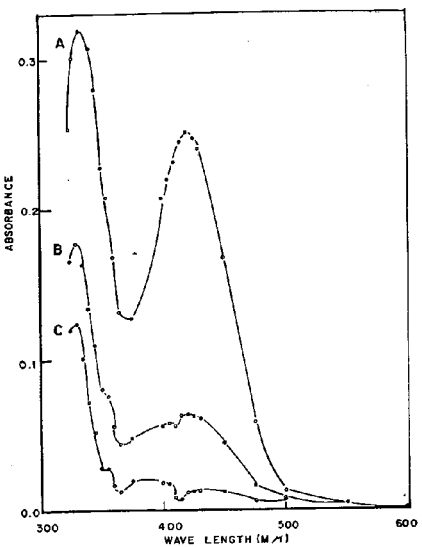


Figure 3. Absorbance curves of toluene solution

A. **25 γ of Se in 10 ml. of toluene**
B. **5 γ of Se in 10 ml. of toluene**
C. **Diaminobenzidine in toluene**
Toluene as blank

No method was mentioned for eliminating the interference from vanadium(V).

In order to find a specific and sensitive reagent for selenium, 3,3′-diaminobenzidine was studied in detail. The results reported here prove that the recommended procedure has the advantages of specificity, sensitivity, and rapidity. The selenium in a solution can be determined within 1 hour. The limit of the sensitivity was found to be 50 p.p.b. (parts per billion) using a 1-cm. cell, and 10 p.p.b. using a 5-cm. cell. In the absence of oxidizing and reducing agents, this method is highly selective.

PROPERTIES OF REAGENT AND ITS SELENIUM COMPOUND

3,3′-Diaminobenzidine is a base, which rapidly darkens in air. Its hydrochloride salt is in the form of white needles, soluble in water. It is most conveniently prepared and is commercially available as the hydrochloride (from J. T. Baker Chemical Co., Phillipsburg, N. J.).

With selenium it forms an intense yellow-colored compound in acid medium below pH 4, but the compound is stable in acid, neutral, and alkaline media. It is quantitatively extracted at pH above 5 but only partially extracted at lower pH values by a few of the common organic solvents.

The reagent is stable in organic solvents but unstable in aqueous solution at room temperature. However, piazselenol is stable in both water and organic solvents. Attempts to stabilize the aqueous solution of the reagent were not successful. It reacts to form an intense yellow coloration with acetone, dioxane, and the organic compounds containing the acetyl group, but forms no coloration with acetic acid.

Reagents and Apparatus. 3,3′-Diaminobenzidine hydrochloride solution, 0.5% in water stored in a refrigerator.

Standard Selenium Solution. A solution containing 1 mg. of selenium per ml. was prepared by dissolving 1.6335 grams of selenous acid (H_2SeO_3) in 1 liter of water. This stock solution was standardized gravimetrically. A 10-p.p.m. selenium solution was prepared by diluting the stock solution.

Formic acid, 2.5*M*.

EDTA Solution, 0.1*M*. The disodium salt of (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid) was used.

Other reagents were of analytical reagent grade. The Beckman Spectrophotometer, Model B, and Beckman pH meter, Model N, were used.

Procedure. Preliminary experiments indicated that the piazselenol could be extracted by toluene. However, the reaction was greatly influenced by acid concentration, temperature, the length of time employed for color development, and the presence of foreign ions. The conditions cited below should be followed carefully in order to obtain reproducible results.

Place an aliquot containing not more than 50 γ of selenium in a 100-ml. beaker. Dilute to approximately 50 ml. with water after adding 2 ml. of 2.5*M* formic acid. Adjust the pH to 2 to 3. Add 2 ml. of 0.5% diaminobenzidine solution and let stand for 30 to 50 minutes. Adjust the pH to 6 to 7 with 7*M* ammonium hydroxide. Transfer to a 125-ml. separatory funnel, add exactly 10 ml. of toluene, and shake vigorously for 30 seconds. Centrifuge the toluene portion for a few minutes. Separate and determine the absorbance at 420 mμ, using a reagent blank. The calibration curves follow Beer's law over the range of 5 to 25 γ of selenium per 10 ml. of toluene at the wave lengths 340 and 420 mμ (Figure 1), 1 to 10 γ of selenium per 6 ml. of toluene (5-cm. cell), and 10 to 100 γ of selenium per 10 ml. of toluene at 420 mμ.

DISCUSSION

Spectral Characteristics. The absorbance curves for the selenium compound in aqueous solution and in toluene are shown in Figures 2 and 3. The curves indicate that piazselenol has absorption maxima at both 340 and 420 mμ, and that the diaminobenzidine was extracted by toluene. Therefore, a reagent

Table II. Effect of Time of Heating on Color Development

Time, Minutes	Absorbance, 420 mμ (2.5 P.P.M. Se)
5	0.253
10	0.250
30	0.252
60	Dark reddish brown color due to decomposition

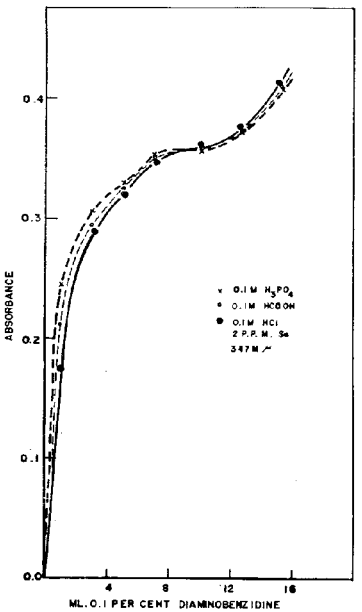


Figure 4. Effect of amount of diaminobenzidine on color intensity of piazselenol

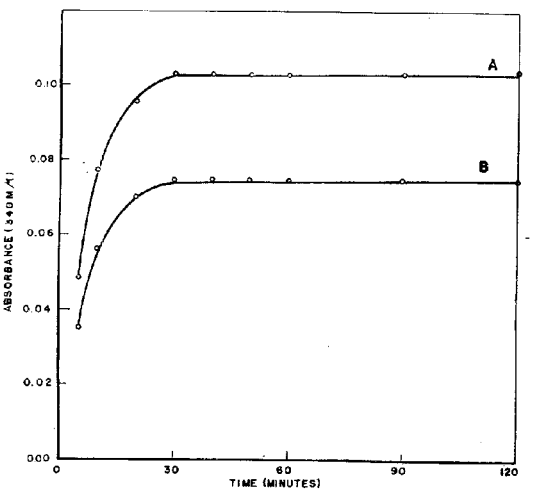


Figure 5. Effect of time of standing on maximum color development of piazselenol

0.1*M* HCOOH
***A*. 15 γ of Se per 25 ml.**
***B*. 10 γ of Se per 25 ml.**

blank should always be used. The wave length of 420 mμ is preferred, as the reagent itself absorbs much less light at 420 mμ than at 340 mμ. When the mixture of diaminobenzidine and selenium was left standing for 30 minutes and then adjusted to a pH above 5, a new absorption maximum was produced wherein the color became more intense.

Specificity. Hoste and Gillis (*3*) have indicated that the yellow color reaction of diaminobenzidine is specific for selenium(IV). However, the oxidizing agents that oxidize diaminobenzidine should be avoided. Moreover, vanadium(V) forms a yellow, reddish brown, or greenish yellow color with the reagent. The interference from iron(III) and copper(II) can be prevented by addition of tartaric acid and oxalic acid, respectively. Furthermore, the measurement of piazselenol in aqueous solution is disturbed by the color of foreign salt.

In this investigation it was found that ethylenediaminetetraacetic acid (EDTA) did not inhibit the formation of piazselenol in acid medium. The use of EDTA prevented the interferences from iron(III), copper, and other metals (except oxidizing agents). The effect of the use of EDTA and toluene extraction on the specificity is listed in Table I.

Effect of Reagents. It was found satisfactory to use 2 ml. of 0.5% diaminobenzidine solution (Figure 4). For less than 5 mg. of metals such as iron or copper 10 ml. of 0.1M EDTA were sufficient. The actual amount of EDTA required for masking or preventing precipitation of polyvalent metals depends upon the amounts of the metals present If EDTA is used to prevent the formation of insoluble hydroxides, it may be added after the piazselenol color has been developed and before the pH is adjusted to 6.

Effect of pH. The color of piazselenol was developed only in the acid medium. pH values between 2 and 3 were found satisfactory. In 2M hydrochloric acid 3 hours and 25 minutes of standing were required for the maximum color development (*3*). Formic acid was preferred, for the amounts of formic acid were not critical. Its concentration from 0.05 to 1.0*M* had no

Table III. Solvent Extraction of Piazselenol

Solvent	Extractability[a]
n-Butyl alcohol	+
Isobutyl alcohol	+
Isoamyl alcohol	+
Benzene	+
Toluene	+
Xylene	+
n-Amyl acetate	+
Chloroform	0
Carbon tetrachloride	0
Isopropyl ether	−
Petroleum ether	−
n-Hexane	−
Cyclohexane	−
Diisobutyl ketone[b]	0
Methyl ethyl ketone	0

[a] + Quantitatively extracted.
0 Partially extracted.
− Not extracted.
[b] Brownish yellow color instead of lemon yellow formed.

Table IV. Comparison of Molar Extinction Coefficient

	pH at Color Development	Se Concn., γ/Ml.	Absorbance	Molar Extinction Coefficient
Aqueous solution				
At 347–340 mμ	1.0	2.5	0.455	28,700
At 340 mμ	2.5	1.0	0.183	28,900
At 420 mμ	6.0	1.0	0.112	17,700
Toluene extraction				
At 340 mμ[a]	6.0	2.0	0.254	20,000
At 420 mμ[a]	6.0	2.0	0.252	19,900

[a] Extracted three times.

effect on the time required for maximum color development (30 minutes).

Time Required for Color Development. The curves in Figure 5 show that a constant absorbance value was obtained after the color had developed at room temperature for 30 minutes. The color could be rapidly developed by heating in a water bath at 100° C. As indicated in Table II, 5 minutes of heating in the water bath produced the maximum color. Longer heating hastened the decomposition of diaminobenzidine. Although the decomposed products were not extracted by toluene, they disturbed the determination in aqueous solution and decreased the concentration of the reagent. As the only advantage of heating was the saving of about 20 minutes, hastening the color development by heating is not recommended under ordinary conditions.

Table V. Determination of Selenium in Presence of Foreign Ions

(Selenium taken, 10.0γ)

Ion	Amount Added, Mg.	Selenium Found, γ
Chromium(III)	5.0	10.0
Molybdenum(VI)	5.0	10.4
Nickel(II)	5.0	10.0
Copper(II)	5.0	10.0
Iron(III)	1.0	9.9
Iron(III)	5.0	10.0
Vanadium(V)	0.1	9.6
Vanadium(V)	0.2	9.8
Vanadium(V)	0.5	10.2
Vanadium(V)	1.0	8.7
Vanadium(V)[a]	5.0	10.6
Tellurium(IV)	1.0	10.2

[a] 4 ml. of 0.5% diaminobenzidine used.

Solvent Extraction. Toluene was used in this investigation. Benzene, isoamyl alcohol, xylene, and amyl acetate were also good solvents. The piazselenol could not be extracted by *n*-hexane and ethers, as indicated in Table III. The curves shown in Figures 6 and 7 show that the pH value required for quantitative extraction of yellow piazselenol was above 5.

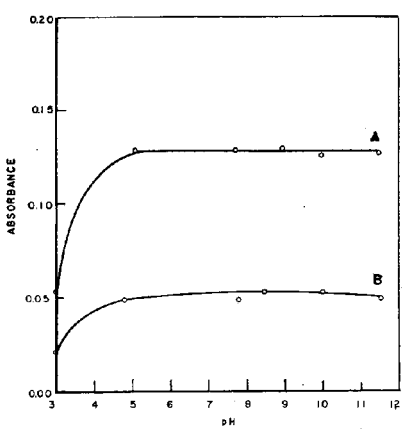


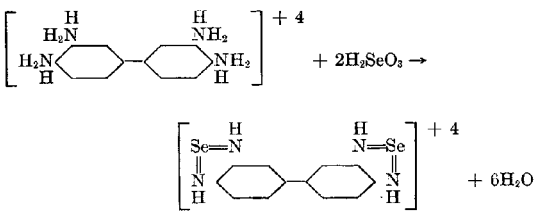
Figure 6. Effect of pH on extraction of piazselenol with toluene

A. 20 γ of Se per 25 ml. of toluene, 420 mμ
B. 10 γ of Se per 25 ml. of toluene, 420 mμ

Erratic results were often obtained when the pH was adjusted to above 10. For simplicity, one extraction with 10 ml. of toluene was used for approximately 50 ml. of solution, although it did not extract 100% of piazselenol, it gave proportional and reproducible results. Two or three extractions may be made, if desired. When one extraction is used, the calibration curve should be made in the same way as the unknown and the volume of the solution to be determined should be carefully controlled.

According to the curves shown in Figure 6, it is evident that the hydrogen ion concentration had important effects upon the extractability of piazselenol into the organic solvent. Furthermore, a structural change possibly took place, because a new absorption maximum at 420 mμ was produced as the pH of the solution was adjusted to neutral or alkaline [see Figure 3 and (*8*), absorption curve.]

One explanation may be proposed for such a change. The reaction between selenium and diaminobenzidine hydrochloride in acid medium may be written as follows:



Acid Medium

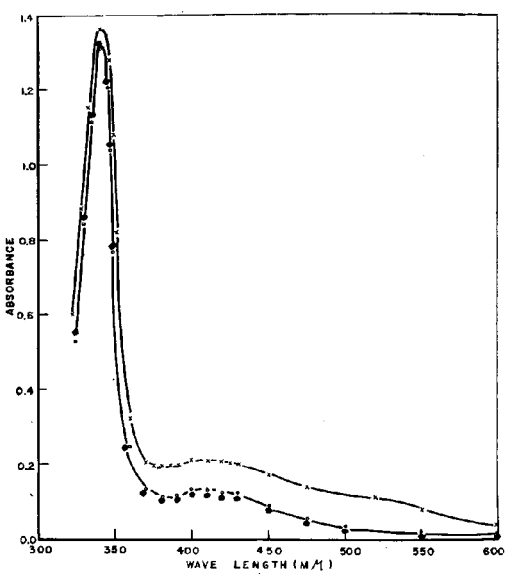


Figure 7. Absorption curves of piazselenol in aqueous solution after development of color in formic acid medium

× pH 10.0
● pH 7.0
○ pH 5.6
Water as blank

Table VI. Determination of Selenium in Arsenic Trioxide and Arsenic

Material	Source	Amount Taken, G.	Selenium, γ Added	Selenium, γ Total found	Se Content, P.P.M.	Recovery, %
Arsenic trioxide	NBS No. 83a	10.0	No	60.0	6.00	...
	Fisher Scientific	10.0	No	2.5	0.25	...
		10.0	10.0	13.0	...	105
	Mallinckrodt	20.0	No	0.78	0.04	...
Super pure arsenic	Westinghouse Chemical Laboratory	15.0	No	4.2	0.28	...
		15.0	10.0	14.5	...	103
Acid mixture (20 ml. HCl + 20 ml. HNO_3)	TransistAR	...	1.0	0.94	...	94

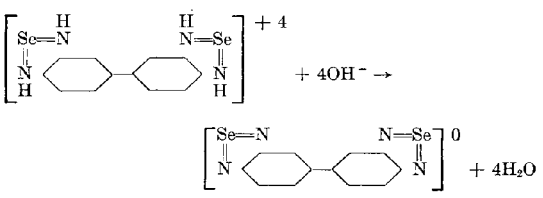
Table VII. Accuracy and Precision

(Selenium added, 10 γ[a])

Selenium Found, γ	Deviation γ	Deviation %	Selenium Found, γ	Deviation γ	Deviation %
10.4	+0.4	+4	9.8	−0.2	−2
10.6	+0.6	+6	10.4	+0.4	+4
9.7	−0.3	−3	10.0	0.0	0
10.0	0.0	0	9.8	−0.2	−2
9.4	−0.6	−6	9.8	−0.2	−2

$$\text{Standard deviation} = \sqrt{\frac{\Sigma (\%\ \text{deviation})^2}{n-1}} = 3.73\%$$

[a] 10 γ of selenium added to 1 g. of arsenic trioxide obtained from Fisher Scientific Co.



Neutral or Alkaline Medium

The number of positive charges on the diaminobenzidine and the piazselenol depends on the pH of the solution and determines the extractability of the piazselenol by toluene; the uncharged molecules are completely extracted into the organic phase, while the extractability of the charged molecules decreases with an increasing number of charges.

Stability of Color. The diaminobenzidine decomposed rapidly in aqueous solution, forming brownish or reddish decomposition products. The piazselenol was stable indefinitely in aqueous solution and toluene.

Molar Extinction Coefficient. Molar extinction coefficient values for piazselenol calculated under various conditions were compared (Table IV). These values decreased at pH 6 as compared to those developed at pH 1.0 or 2.5.

Interference Studies. Iron(III), copper(II), vanadium(V), and other oxidizing agents interfere in the method. No interference was found if iron and copper were complexed by EDTA at pH 2 to 3. Although vanadium(V) oxidized diaminobenzidine, traces of vanadium did not affect the results. With 5 mg. of vanadium, no interference occurred if 4 ml. of 0.5% diaminobenzidine were used. From the practical point of view, the tolerable amounts of vanadium are still limited. Fluoride was ineffective in masking vanadium. If vanadium(V) was reduced by ascorbic acid, diaminobenzidine did not seem to be oxidized. However, ascorbic acid inhibited the formation of piazselenol. The addition of sodium azide had no inhibitive effect. The interference of chromium(III), molybdate, nickel, tungstate, copper, cobalt, and titanium, due to their own color, could be effectively eliminated by the addition of EDTA and the extraction technique. As the majority of the common ions do not interfere, as indicated by Hoste and Gillis (*3*), only the results for interfering ions mentioned by them are listed in Table V. It may be concluded that only the substances which either oxidize diaminobenzidine or reduce selenite to elemental selenium interfere in this method. In their absence the method is highly selective. It can tolerate large amounts of salt, as demonstrated by the results in Table VI.

DETERMINATION OF SELENIUM IN ARSENIC

There has been a demand for a sensitive analytical method of determining traces of selenium in "super pure" arsenic, which has potential use for making semiconductors.

Samples of 10 to 20 grams of elemental arsenic or arsenic trioxide were dissolved in a minimum amount (20 ml.) of 1 to 1 hydrochloric acid and nitric acid under a hood. The reaction between elemental arsenic and the acid mixture is violent; therefore, the acid mixture should be carefully added a few milliliters at a time in order to avoid splattering. The mixture may be gently warmed, if necessary. After the solution was complete, it was evaporated to near dryness with care to avoid baking. The white substance was dissolved by addition of 40 to 45 ml. of water with the aid of gentle heating. The solution was cooled to room temperature. After addition of 2 ml. of 2.5*M* formic acid and 2 ml. of 0.5% diaminobenzidine hydrochloride solution, the mixture was adjusted to pH 2.5 with ammonium hydroxide. After 30 minutes of standing, the solution was adjusted to pH 6 and extracted with 10 ml. of toluene. The toluene extract was read at 420 mμ using a reagent blank.

The results in Table VI indicate that the National Bureau of Standards arsenic trioxide contained much more selenium than the analytical reagent grade arsenic trioxide from Mallinckrodt and the Fisher Scientific Co.

Accuracy and Precision. The recovery of known amounts of selenium added to the arsenic samples was satisfactory, as indicated in Tables V, VI, and VII. Precision of the method was tested by addition of 10.0 γ of selenium to 1 gram of arsenic trioxide (Table VII). A standard deviation of 3.73% was obtained.

The proposed procedure has also been successfully applied to determination of traces of selenium in stainless steel and copper. The results will be published elsewhere.

ACKNOWLEDGMENT

The author is indebted to J. Pien and J. Hoste for providing diaminobenzidine and to Shirley Brown for aid in obtaining some of the data reported.

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RECEIVED for review June 8, 1956. Accepted July 31, 1956. Division of Analytical Chemistry, 130th Meeting, ACS, Atlantic City, N. J., September 1956.

Colorimetric Method for Determination of Glucosamine and Galactosamine

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A colorimetric method is presented for the determination of the hexosamines which is based upon their conversion to the *N*-acetyl derivatives. Because *N*-acetylglucosamine and *N*-acetylgalactosamine do not yield the same color intensities when treated under standard conditions, it is possible to utilize the procedure for the analysis of mixtures of the two sugars.

THE implication of the mucopolysaccharides and mucoproteins in many biological phenomena has led to renewed interest in analytical methods for the determination of these substances and of their constituents. The most characteristic components of the polymers are the hexosamines, glucosamine (2-amino-2-deoxy-D-glucose) (I) and galactosamine (2-amino-2-deoxy-D-galactose) (II), which generally occur as their respective *N*-acetyl derivatives, *N*-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) (III) and *N*-acetylgalactosamine (2-acetamido-2-deoxy-D-galactose) (IV).

Quantitative analysis of the mucoid substances is frequently performed by acid hydrolysis to I and/or II, which are then determined colorimetrically. The colorimetric method commonly employed is the classical Elson-Morgan method or one of its recent modifications (*2–4, 9, 17*). The problem of the determination of I and II in mixtures has received much attention within recent years because such mixtures are common in biological materials. Some of the methods which have been suggested involve paper chromatography, ionophoresis, ion exchange chromatography of derivatives, and the like (*10*).

This paper describes a simple colorimetric method which exhibits advantages in accuracy and specificity over the classical Elson-Morgan method for determining either I or II separately, and, when combined with the Elson-Morgan method, permits determination of I in the presence of II, or vice versa. It is based on the following principles: (*a*) The acetylated hexosamines (III and IV) produce color when treated first with alkali and then with Ehrlich's reagent—i.e., omitting the acetylacetone step in the classical Elson-Morgan procedure (*12, 21*); (*b*) quantitative *N*-acetylation of I and II to III and IV, respectively, can be performed with acetic anhydride in aqueous solution (*15*); (*c*) in the Elson-Morgan procedure, I and II yield equal amounts of color, whereas with the acetic anhydride technique, the corresponding acetylated derivatives differ in chromogenic properties, IV yielding only 23% of the color obtained with III (*1*). Application of the techniques to several polymers of biological origin has yielded results which agree within experimental error with those obtained with an independent method of determination.

Concomitant with submission of this paper, a publication appeared by Reissig, Strominger, and Leloir (*13*), which reported a colorimetric method for the estimation of *N*-acetylamino sugars. The procedure was a modification of that of Aminoff, Morgan, and Watkins (*1*) and was used, among other applications, for the analysis of mixtures of III and IV.

MATERIALS

Elson-Morgan Procedure. The reagents, including the ion exchange resin, are prepared as described by Boas (*3*). The columns are prepared from borosilicate glass test tubes (10 × 75 mm.) which are pulled out at the tips so that they resemble medicine droppers. Glass wool is placed in the tips and 1.0 ml. of resin suspension (0.5 ml. of resin) is added to each tube. The glass wool is packed so that solutions pass through the columns at the rate of 0.1 ml. per minute. If necessary, a slight positive air pressure is used to force the liquid through the resin.

Acetic Anhydride Procedure. The acetic anhydride reagent is prepared just before use by diluting 5.0 ml. of reagent grade acetic anhydride to 100 ml. with ice water. The flask is kept in ice during the pipetting procedure.

The Ehrlich reagent for the acetic anhydride procedure differs from that used in the Elson-Morgan method in the use of acetic acid rather than ethyl alcohol as the solvent. Commercial *p*-dimethylaminobenzaldehyde (Matheson, No. 1660) was frequently found to be as effective as the purified material. When the reagent blank is yellow, the reagent must be purified (*5*). The reagent (0.360 gram) is weighed into a 50-ml. volumetric flask and stored in the dark. Just before use, the solid is dissolved in approximately 20 ml. of glacial acetic acid followed by 0.5 ml. of concentrated hydrochloric acid. The mixture is then diluted to the mark with glacial acetic acid.

Sodium bicarbonate solution, saturated.

Sodium carbonate, 1.0*N* solution.

Glucosamine hydrochloride (Pfanstiehl) is purified by recrystallization from aqueous ethyl alcohol or by an ion exchange technique (*15*).

Galactosamine hydrochloride and the anomeric phenyl *N*-acetyl-D-glucosaminopyranosides are prepared by the reported methods (*14, 15*).

The acetylation tubes are glass-stoppered, 2.0-ml., volumetric tubes (Corning No. 5640).

Sodium chondroitin sulfate and sodium hyaluronate were prepared by the reported methods (*11, 16*).

PROCEDURES

Hydrolysis of Hexosamine-Containing Polymers. Where the samples contain I and II in combined form, hydrolysis is necessary prior to the determination. The samples (solid or solution) are placed in borosilicate glass test tubes with sufficient concentrated hydrochloric acid solution to make the final mixture 4*N* with respect to the acid. The tubes are sealed, fully immersed in a boiling water bath, and generally maintained at 100° C. for 5 hours. The time of hydrolysis and the concentration of acid used for optimal yields of hexosamine are functions of the particular compounds under investigation (*3*). The tubes are cooled by immersion in a water bath so that the levels of liquid inside and outside the tubes are the same. The tubes are then opened and placed in a vacuum desiccator over calcium chloride and soda lime. The removal of hydrochloric acid and all subsequent steps in the analysis must be performed as rapidly as possible. The desiccators are evacuated with an oil pump to 10^{-3} mm. of mercury or lower. During the evacuation procedure bumping occurs if the tubes are too narrow. Under the conditions described, 40 tubes (each containing 3 ml. of hydrolysate) in a single desiccator are dried completely after standing overnight.

If only the acetic anhydride technique described below is to be performed, the dried samples are dissolved in water and 1.0-ml. aliquots are transferred to the 2.0-ml. acetylation tubes.

Determination of Total Hexosamine (Elson-Morgan Procedure). The samples obtained after hydrolysis and drying are dissolved in 1.0-ml. portions of water and transferred to the ion exchange columns. The liquid is allowed to drain through the resin beds; when the meniscus has dropped below the surface of the resin, the hydrolysis tubes are washed with water and the washings are added to the respective columns. The hydrolysis tubes are washed three or four times each with 2.0-ml. portions of water. In each case, the wash liquid is permitted to drain through the column completely before the addition of the subsequent portion. The entire sample of hexosamine should now

be on the resin, and the column adequately washed. With large samples, the washing process is performed more thoroughly. Elution is accomplished with 0.50*N* hydrochloric acid solution, which is added in 1.0-ml. portions to the tops of the columns in the same manner as described for the washing procedure. The eluates are collected in 5.0-ml. volumetric flasks and the process is stopped when the flasks are full. The eluates (*E*) now contain all of the hexosamine originally placed in the hydrolysis tubes.

For the acetic anhydride procedure, aliquots of *E* are placed in test tubes, dried in vacuo, and dissolved in 1.5 ml. of water. Samples (1.0 ml.) are transferred to the 2.0-ml. acetylation tubes and treated as described below. This method requires from 25 to 350 γ of I and from 100 to 1400 γ of II.

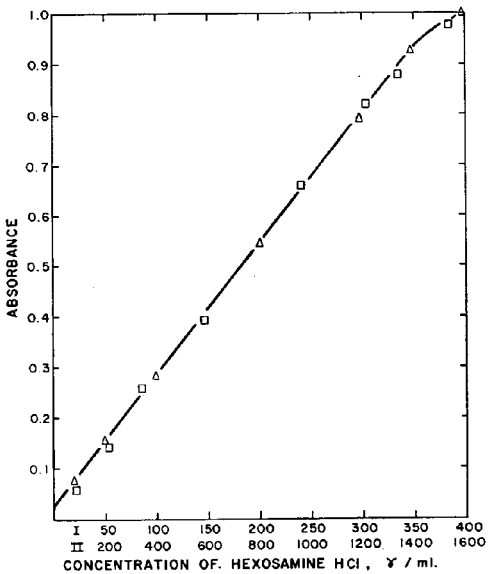

Figure 1. Relationship of absorbance to concentration of hexosamine hydrochlorides

△ = I-glucosamine
□ = II-galactosamine

For the Elson-Morgan procedure, samples of *E* containing between 4 and 24 γ of hexosamine are placed in Pyrex screw-capped test tubes (Corning No. 9825; 16 × 150 mm.). The samples are dried in vacuo and 1.0 ml. of water is added to each tube. After the addition of 1.0 ml. of acetylacetone reagent, the tubes are closed with the caps, which have had the liners replaced with Teflon to resist the action of the acetylacetone. The remainder of the procedure is carried out as described (*3*) with the exception of the volumes of ethyl alcohol (4.0 ml.) and Ehrlich reagent (1.0 ml.).

It is imperative that standard curves be obtained with each set of unknowns and that the standards be treated in the same manner as the unknowns.

Determination of I or II by Acetic Anhydride Procedure. Either the samples, standards, water, or suitably prepared aliquots of eluate (*E*) are placed in the acetylation tubes (1.0 ml. of solution), followed by 0.1 ml. of acetic anhydride reagent and 0.1 ml. of saturated sodium bicarbonate solution. The solutions are mixed and allowed to stand at room temperature for 10 minutes. The excess anhydride is destroyed by placing the tubes in boiling water for 3 minutes. After cooling, 0.5 ml. of 1.0*N* sodium carbonate solution is added to each tube and the solutions are vigorously mixed. The tubes are then placed in boiling water for exactly 3 minutes, removed, and cooled in tap water. The solutions are adjusted to 2.0 ml. with distilled water, and the tubes are stoppered and inverted several times. Aliquots

Table I. Analysis of Mixtures of I and II

Ratio of II to I in Mixture	Concentration of II, γ/Ml. Added	Found	% Error
	Unhydrolyzed		
0.1	15	15	0
0.2	32	30	6.3
0.4	55	60	9.1
0.5	76	75	1.3
0.6	92	90	2.2
0.8	120	120	0
0.9	138	135	2.2
	Hydrolyzed[a]		
0.1	18	19	5.5
0.2	36	36	0
0.4	72	64	11
0.5	90	91	1.1
0.6	108	111	2.7
0.8	144	147	2.1
0.9	162	167	3.1

[a] Hydrolysis was carried out in 4*N* hydrochloric acid solution for 5 hours followed by ion exchange purification as described in text.

of 1.0 ml. are transferred to colorimeter tubes, followed by the addition of 5.0 ml. of glacial acetic acid with shaking. Then 5 ml. of Ehrlich reagent (prepared for the acetic anhydride method) is added to the tubes at 30-second intervals (by stop watch) and the tubes are shaken vigorously during the addition. The color is allowed to develop and the absorbance of each solution is determined exactly 30 minutes after the addition of the last reagent. With the Evelyn colorimeter, the No. 530 filter is used. Although the color is fairly stable, there are slight changes with time, and the practice of reading the tubes in the manner indicated avoids this source of error. It is advisable to determine the optimal time with new batches of *p*-dimethylaminobenzaldehyde. Color development is influenced by inorganic salts, so that the concentrations of ions in the standard solutions must be the same as in the unknowns.

Calculations for Mixtures of I and II. Aliquots of the eluate are analyzed by both the Elson-Morgan and acetic anhydride procedures. The total hexosamine (*H*) concentration of the unknown is obtained from the Elson-Morgan procedure, because I and II yield equivalent absorbances at equivalent concentrations. With the acetic anhydride procedure an absorbance is obtained for the unknown mixture of I and II ($A_{mixt.}$) and at the same time standard curves of absorbance *vs.* concentration are obtained for known samples of I and II. The following data are therefore available from the acetic anhydride procedure: $A_{mixt.}$, B_1 and B_2 (slopes of standard curves of I and II, respectively), and Y_1 and Y_2 (intercepts of standard curves on absorbance axis). Absorbance is related to concentration as follows:

$$A_1 = B_1\mathrm{I} + Y_1$$

$$A_2 = B_2\mathrm{II} + Y_2$$

where A_1 is the absorbance for any concentration of I and A_2 the absorbance for any concentration of II. The absorbances of I and II in a mixture are additive; therefore, the absorbance of the unknown

$$A_{mixt.} = A_1 + A_2 = B_1\mathrm{I} + Y_1 + B_2\mathrm{II} + Y_2$$

The total hexosamine concentration (*H*) of the unknown mixture is

$$H = \mathrm{I} + \mathrm{II}$$

Simplification yields $A_{mixt.} = B_1\mathrm{I} + Y_1 + B_2(H - \mathrm{I}) + Y_2$

or

$$\mathrm{I} = \frac{A_{mixt.} - (Y_1 + Y_2) - B_2H}{B_1 - B_2}$$

Examination of the final equation indicates that the concentration of I in the unknown mixture can be determined by a combination of the Elson-Morgan method (which yields the value, *H*) and the acetic anhydride method (which yields all the other data). For the most accurate analyses, the slopes and intercepts of the standard curves are derived from the data by the method of least squares. It was generally found that the intercepts were

small enough to be disregarded and that the slopes of the curves could be obtained by simple graphic interpolation. With the latter technique, the right side of the last equation reduces to a simple fraction. It should again be stressed that the method is a differential one, and is therefore subject to certain errors—i.e., the minor component is measured less accurately than the one present in higher concentration.

RESULTS

Standard Curves. Typical standard curves of absorbance *vs.* concentration of I and II obtained with the acetic anhydride method are shown in Figure 1. There are small variations from day to day, particularly in the intercepts. As is apparent from the curves, II must be present at about four times the concentration of I to yield the same absorbance. Boas (*3*) has indicated that the useful range of concentration in the Elson-Morgan procedure is such that the ratio of highest to lowest concentration is 3 to 1. More generally, other workers have extended this to approximately 5 or 6 to 1. In the acetic anhydride method, as indicated in Figure 1, the ratio is approximately 17 to 1. It should be noted that the acetic anhydride method is considerably less sensitive than is the Elson-Morgan method.

Specificity. When the *N*-acetylhexosamines, III and IV, were analyzed directly without hydrolysis, they yielded the expected absorbances. This indicates that the acetylation step is quantitative under the conditions described above.

Several investigators (*7*, *8*) have noted the lack of specificity of the Elson-Morgan method. The most troublesome artifacts are the spurious colors obtained when mixtures of neutral sugar and amino acids are treated under the standard conditions. The hexosamines, neutral sugars, and amino acids are frequently found together as components of large molecules—i.e., the mucoproteins. When a commercial casein hydrolyzate or a mixture of lysine and fructose was analyzed by the Elson-Morgan procedure, the expected pink colors developed. Aliquots of the same mixtures yielded no color with the acetic anhydride method. A number of mold extracts have been found to yield considerable color with the Elson-Morgan procedure (no prior resin treatment) but exhibited none with the acetic anhydride technique.

Accuracy. A measure of the accuracy of the acetic anhydride method was obtained by performing a series of analyses with standard solutions of phenyl *N*-acetylglucosaminopyranoside. In this case, it is necessary to hydrolyze the glycoside with acid before the colorimetric method can be applied. A series of samples ranging in concentration from 40 to 600 γ yielded recoveries of 100 $\pm$ 3%.

Mixtures of I and II. Table I presents the results obtained with various simple mixtures of I and II, as well as those obtained when the mixtures were first treated under usual conditions of hydrolysis, drying, resin adsorption, and the like. While the component present in highest concentration can be determined satisfactorily, this is not always true for the minor component, especially when it is 10% or less of the mixture.

Mixtures of Hyaluronic Acid and Sodium Chondroitin Sulfate. These mucopolysaccharides are frequently found together, and the problem of separation, purification, and analysis has received much attention in recent years. Because hyaluronic acid contains I, and chondroitin sulfate contains II, it appeared possible to analyze mixtures of the two polysaccharides by hydrolysis to the hexosamines and application of the Elson-Morgan and acetic anhydride procedures described above. The results obtained with artificial mixtures are presented in Table II. Again, the accuracy of the method for either component is shown to be a function of the relative concentration of that component.

Table II. Analysis of Mixtures of Sodium Chondroitin Sulfate and Sodium Hyaluronate

Ratio of CSA/HA[a] in Mixture	Concentration of CSA, γ/Ml.		Concentration of HA, γ/Ml.	
	Added	Found	Added	Found
0.15	121	130	684	675
0.35	242	271	449	420
0.52	343	304	322	361
0.68	604	624	286	266
0.70	607	610	264	261
0.90	970	927	106	149

[a] CSA, sodium chondroitin sulfate; HA, sodium hyaluronate.

Application to Some Natural Products. Results of analyses of several typical substances containing both I and II are shown in Table III. To make the analyses comparable to those obtained in other laboratories, nitrogen determinations were performed upon aliquots of the solutions of the mucoid substances, and the ratios of hexosamine to nitrogen are also presented. The absolute hexosamine contents are not considered reliable because the moisture content of these substances can vary considerably, and it is difficult to remove the water completely.

Table III. Analysis of Mucoprotein Samples

Sample	Source	Total Hexosamine/N Ratio, μmole/-μmole $\times$ 100	Fraction of Total Hexosamine, %	
			Glucosamine	Galactosamine
Mucoprotein I[a]	Tonsils	10.7	19.3	80.7
Orosomucoid[b]	Human plasma	8.75	71.4	28.6
Fraction VI	Human plasma	3.80	61.5	38.5
Fraction VI	Bovine plasma	6.35	66.6	33.4
Ovomucoid[c]	Hen's eggs	8.25	68.5	31.5
Polysaccharide "A"[d]	Hog mucin		98+	2 (?)
Purified intrinsic factor		9.90	51.5	48.5

[a] Prepared according to (*6*).
[b] Prepared according to (*19*).
[c] Prepared according to (*20*).
[d] Prepared according to (*18*).

Table IV. Analysis of Blood Group Substance Samples by Two Methods

Preparation	Chromatographic Method (*10*), % of Total Hexosamine		Differential Colorimetric Method, % of Total Hexosamine	
	Glucosamine	Galactosamine	Glucosamine	Galactosamine
Hog 23	72	28	71	29
Hog 42 4%	69	31	72	28
PM OH insol.	74	26	71	29
Horse 5 25%	50	50	56	44

Application to Blood Group Substances. To compare the results obtained by the procedures described here with those obtained by another technique, samples were supplied which had been analyzed by the method described by Leskowitz and Kabat (*10*). This procedure is an absolute method because the hexosamines are reduced to the hexosaminitols, converted to the dinitrophenyl derivatives which are separated by chromatography, and then analyzed colorimetrically. The results obtained with the two procedures are presented in Table IV. The agreement between the methods is better than predicted in view of the errors inherent in both techniques, particularly in the differential method presented in this report.

CONCLUSIONS

The need for a simple colorimetric procedure for the analysis of mixtures of I and II for routine work is evident from the variety of methods described in the recent literature. While the technique described here is a differential colorimetric method

and is, therefore, subject to the limitations discussed above, the agreement between the observed and expected values for mixtures of I and II, of hyaluronic acid and chondroitin sulfate, and of the blood group substance preparations indicates that the method is valid within certain limits. Within the errors indicated, therefore, the analyses of the natural products offered in Table III are considered reliable. The procedure has also been utilized where mixtures of I and II were possibilities. Thus, in a survey of chitin obtained from 25 strains of fungi, the results obtained with the acetic anhydride and Elson-Morgan procedures agreed within experimental error, indicating that II was not present in significant quantities in the samples.

ACKNOWLEDGMENT

The authors are deeply indebted to Eugene Hess, Richard Winzler, H. F. Deutsch, Harry Smith, and William Williams, who supplied the mucoprotein samples, and to Elvin Kabat for providing the samples and analyses which were necessary for cross-checking the methods described with the chromatographic method. The editorial assistance and suggestions offered by William D. Robinson are also acknowledged.

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RECEIVED for review January 3, 1956. Accepted July 3, 1956. The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies of the University of Michigan. This investigation was supported in part by grant A-512 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and a grant from the Michigan Chapter, Arthritis and Rheumatism Foundation.

Estimation of 1-Butanol and Ethyl Cellosolve in Waste Streams with Sudan III Reagent

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The acid-base indicator properties of Sudan III [1-(*p*-phenylazo)-phenylazo-2-naphthol] together with its negligible solubility in water permit its use in a qualitative and estimative test for 1-butanol and ethyl Cellosolve. This method is applicable to the determination of these solvents in dilute aqueous mixtures, where each does not exceed 1%. In this range the test is accurate to ±0.2% of each solvent.

AT THE present time no reliable and simple qualitative and estimative method of analysis is reported for 1-butanol and ethyl Cellosolve (2-ethoxyethanol) in dilute aqueous mixtures. The need for such a test has become evident in recent years, when increased emphasis has been placed on close control and the reduction of the biochemical oxygen demand (B.O.D.) of waste streams and plant effluents. The determination should measure both solvents separately in a mixture of the two, it should have high reproducibility and fair accuracy, and it should be relatively insensitive to other waste stream constituents. These criteria are met by the Sudan III method.

Sudan III, 1-(*p*-phenylazo)-phenylazo-2-naphthol, an important fat stain, is a water-insoluble, weakly acid dye that exhibits interesting acid-base indicator properties. Its color changes from red to violet in inorganic aqueous bases at pH 10 or greater when it is solubilized by some water-miscible organic solvent. Under the conditions of the Sudan III method described below, ethyl Cellosolve solubilizes Sudan III in the base, whereas butanol does not. The absorption spectra of the red and violet forms of Sudan III in Cellosolve (Figure 1) show the bathochromic and hypochromic shifts produced by base in the visible absorption maximum.

Some organic bases also cause Sudan III to change color. It appears from the data in Table I that the dye is sensitive to normal primary aliphatic amines up to *n*-butylamine, and to dimethylamine. Significant interference by these bases is avoided by distilling the alcohols from a strongly acidified solution. Solid fatty acids which may distill over, or entrained suspended solids, constitute a second type of interference which obscures the surface of the reagent during the analysis. These are removed by chilling and filtering the distillate.

The Sudan III used is a commercial grade (National Aniline Division, Allied Chemical and Dye Corp.) certified by the Biological Stain Commission. Trubey and Christman (*3*) chromatographed commercial Sudan III into its components. Werner and Christman (*4*) found the fat-staining property to be due to the orange impurity and not to Sudan III. This orange stain was later isolated and characterized by Christman and Cunningham (*1*). In the belief that the acid-base indicator property might also be due to an impurity rather than to the Sudan III, the commercial product was chromatographed in

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Table I. Sudan III as Acid-Base Indicator in Bases

pKb (2)	Red	Violet	pKb (2)	Red	Violet
2.80	Piperidine		4.07		Ethylenediamine
2.90	Diethylamine		4.17	*p*-Phenylethylamine	
3.09	Dipropylamine		4.28	Trimethylamine	
3.25		Ethylamine	4.44	*p*-Methoxybenzylamine	
3.25	Triethylamine		4.56		Ethanolamine
3.29		Dimethylamine	4.70	Benzylamine	
3.30	Tripropylamine		4.77	*o*-Methylbenzylamine	
3.39		*n*-Butylamine	8.80	Phenylhydrazine	
3.44	*sec*-Butylamine		9.42	Aniline	
3.55	*tert*-Butylamine		10.08	1-Naphthylamine	

this laboratory according to the method of Trubey and Christman (3). The different fractions were then tested for indicator properties, and their absorption spectra in acid and base compared with those obtained with the commercial product. The Sudan III fraction gave the color change, and the spectra obtained were identical in characteristics to those of the commercial product. Neither the orange nor any other fraction showed these properties.

PROCEDURE

Reagents. Prepare the standard solutions of the solvents using reagent grade 1-butanol and ethyl Cellosolve, so that a complete set includes each solvent individually at 0.2% increments to 1% w./v., and in mixtures not exceeding 1% w./v. of either solvent.

Prepare Sudan III reagent by shaking 0.02 gram of Sudan III, of certified dye content $\geq$ 90%, with 200 grams of dried reagent grade sodium chloride crystals until they are uniformly coated with dye. To this add 500 grams of 20- to 40-mesh anhydrous reagent grade potassium carbonate and roll or shake the mixture until it is uniform. Protect this reagent from moisture, for it will absorb water from the air and become less sensitive.

Method. Distill a mixture of 10 ml. of the sample and 10 ml. of 2*N* sulfuric acid. Collect the first 10 ml. of distillate and filter if necessary.

Place 7 grams of the Sudan III reagent in a 10-ml. beaker. Pipet 2 ml. of the distillate over the reagent so as to wet it uniformly. As the distillate soaks in during the pipetting, note any color change in the wetted reagent and observe the color and extent of extraction of the dye. Stir the mixture slowly with a thin stirring rod, and again observe the color and extent of extraction of Sudan III. Compare these observations with those using standard solutions, and with the criteria set forth in Table II. Mixtures of butanol, Cellosolve, and water with high solvent content may appear to give a low Sudan III test. If the initial determination yields 0.2% or less, add 2 ml. of distilled water to the wet reagent and stir. If 0 to 0.2% of solvent is actually present, no increase in dye extraction occurs. If high percentages are present, pronounced extraction will be observed. When it is necessary to estimate percentages of either solvent that are greater than 1%, the distillate may be diluted with distilled water to obtain an approximate determination. This over-all dilution is then made on the original sample and it is reanalyzed.

DISCUSSION

Sudan III Reagent. The potassium carbonate performs three functions. It is the base that effects the color change in Sudan III when Cellosolve is present. It salts out the organic phase. Finally, it minimizes the solubility of aqueous base in the organic phase, so that the test is made less vulnerable to interference by other water-miscible solvents, such as methanol. Uniformity of particle size is necessary to ensure reproducible results. The sodium chloride merely acts as a carrier for the uniform distribution of the Sudan III.

Estimations. It is possible with experience to differentiate between samples 0.2% apart (Table III). It appears that butanol and Cellosolve act independently in extracting the dye, thus making the test useful in determining mixtures of the two. However, as this method depends upon the extraction of dye and the mutual solubility of Sudan III and base in the organic phase,

Table II. Criteria for Analysis with Sudan III

1-Butanol	%	Ethyl Cellosolve
No extraction or color change	0.0	No extraction or color change
Little dye extracted, red, disappears on mixing	Trace–0.2	Mixture turns violet with no noticeable extraction of dye
Definite extraction of red globules stable on mixing	0.2–0.5	Violet "dots" extracted, stable on mixing
Large red droplets extracted	0.5–1.0	Large violet droplets extracted

Table III. Accuracy in Estimations Using Sudan III Method

Present, %[a]		Found, %[b]		Present, %		Found, %	
BuOH	Cellosolve	BuOH	Cellosolve	BuOH	Cellosolve	BuOH	Cellosolve
1.0	1.0	1.0	1.0	0.2	0.2	0.2	0.2
0.0	1.0	0.0	0.8	1.0	0.0	1.0	0.0
0.5	0.0	0.5	0.0	0.0	0.2	0.0	0.2
0.5	0.5	0.5	0.5	0.0	0.4	0.0	0.5
0.0	0.5	0.0	0.4	0.0	0.8	0.0	0.8

[a] Each mixture prepared using solvents in distilled water and in plant effluents free of solvents.
[b] Identical results obtained for water and effluent solutions.

solvents other than butanol and Cellosolve may yield positive tests. Table IV reports the results when a number of known aqueous samples of common solvents were prepared using distilled water and stripped plant effluents, then coded, scram-

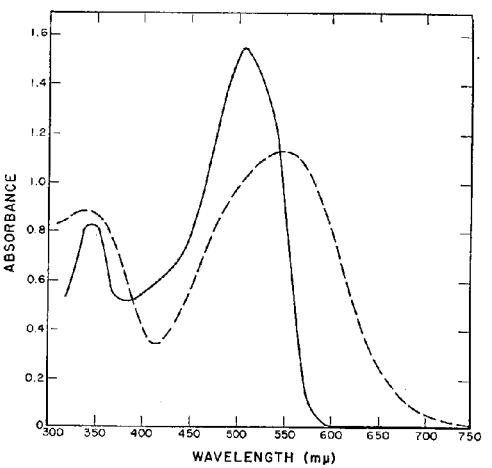

Figure 1. Absorption spectra of red and violet forms of Sudan III

—— Red form. $5 \times 10^{-5} M$ Sudan III in Cellosolve
— — Violet form. $5 \times 10^{-5} M$ Sudan III in Cellosolve, 0.05 *M* in NaOH

Table IV. Interference of Other Solvents in Sudan III Method

Other Solvent	%	Apparent % Found BuOH	Apparent % Found Cellosolve	Other Solvent	%	Apparent % Found BuOH	Apparent % Found Cellosolve
Methanol	10.2	0.0	0.5	2-Propanol	10.0	1+	0.0
	2.0	0.0	0.0		2.0	0.2	0.0
					1.0	0.0	0.0
Ethanol	10.0	0.0	1+	Acetone	10.0	0.0	1+
	2.0	0.0	0.2		2.0	0.0	0.2
	1.0	0.0	0.0		1.0	0.0	0.0
1-Propanol	10.0	1+	0.2				
	2.0	0.2	0.2	Dioxane	10.0	1+	0.0
	1.0	0.0	0.0		2.0	0.2	0.0
					1.0	0.0	0.0

bled, and analyzed according to the procedure above. The only solvents that gave a positive test at concentrations of 1% or less were butanol and Cellosolve.

Advantages. The Sudan III method is sensitive and reproducible to ±0.2%. Moreover, a complete analysis may be run in 10 to 15 minutes. Little interference is encountered from other solvents when they are present in percentages comparable to butanol and Cellosolve. Finally, no added difficulties were encountered in analyzing over 10,000 waste stream samples by this method, when dissolved salts, amines, and oils were present. Reproducibility of this test in the hands of laboratory personnel has been confirmed by hundreds of reruns, which almost invariably yielded identical results.

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RECEIVED for review December 22, 1956. Accepted July 23, 1956. Meeting in-Miniature, New York Section, ACS, New York, N. Y., March 1956.

Semiquantitative Specific Test Paper for Glucose in Urine

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Data are presented to show that a test paper consisting of the enzymes glucose oxidase, catalase, and horseradish peroxidase, F.D.C. yellow No. 5 dye, and *o*-tolidine, when made from standardized enzymes, is 96% accurate in the range from 0 to 2% glucose. The paper is dipped into the specimen, removed, and compared after 1 minute to a color chart. Results are not influenced by the normal variation of pH and temperature of the urine, or by the presence of common drugs or metabolites in the urine. This test is more sensitive and more selective for the lower concentrations of glucose than is the Benedict's test. The paper is stable if protected from light.

THE use of paper as a medium for analytical reagents has been accelerated with the routine use of filter paper chromatography and electrophoresis and with the availability of general literature references on spot tests in the text by Feigl (*3*). A paper test for glucose in urine, which would have a simplicity and accuracy similar to the paper for pH measurements (Hydrion paper, Micro Essential Laboratories, Brooklyn 10, N. Y.), has been desired for many years. However, the measurement of the nonspecific reducing properties of glucose by the usual chemical reagents coated on paper has not been practical because of instability of such reagents on paper under normal storage conditions.

Keston (*6*) has described a novel idea for the simultaneous use of two enzymes for testing for glucose. The reactions involved are:

$$\text{Glucose} + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + o\text{-tolidine} \xrightarrow[\text{peroxidase}]{\text{horse-radish}} \text{blue color}$$

Table I. Medications Used for Diabetic and Nondiabetic Patients under Test

Patient	Medication
1	Liver extract
2	Vitamin B_{12}
3	Insulin, digitoxin
4	Diethylstilbestrol
5	Aspirin, phenobarbital
6	Aspirin, phenacetin, caffeine
7	Digitoxin, ethinamate
8	Reserpine, lactose, protoveratrine
9	Aspirin, ethinamate, 3-*o*-toloxy-1,2-propanediol
10	Tricyclamol sulfate, multiple vitamins, aspirin, phenacetin, caffeine, ethinamate, dioxyline
11	Ethinamate, folic acid
12	Insulin, chloral hydrate, quinidine
13	Lente insulin, digitoxin
14	Aspirin (arthritis dosage)
15	Secobarbital, quinine sulfate
16	Tricyclamol, magnesium trisilicate, aluminum hydroxide, reserpine
17	Theamin
18	Carbacrylamine resin, thenylpyramine
19	Digitoxin, multiple vitamins, erythromycin
20	Cortisone

Glucose oxidase (*2*) is a specific enzyme that catalyzes the oxidation of glucose with oxygen into gluconic acid and hydrogen peroxide. The hydrogen peroxide formed in the presence of *o*-tolidine and the enzyme horse-radish peroxidase forms a blue color that has not been identified.

Glucose oxidase has been used previously for the manometric determination of glucose in biological materials by Keilin and Hartree (*4*) and for the titrimetric determination of glucose in corn sirup by Whistler and coworkers (*8*). The mechanism of the action of glucose oxidase is amply discussed by Bentley and Neuberger (*1*), and the specificity by Keilin and Hartree (*5*). The isolation and properties of horse-radish peroxidase are reported by Theorell (*7*).

Work was started in this laboratory to develop a test paper for glucose based on the reactions described by Keston (*6*), that would

Table II. Statistical Study of the Accuracy of Glucose Test Paper

Reported	% Reported Observations[a]				
	% Glucose Present				
	0	0.10	0.25	0.50	2
0	100				
0.10		97	1		
0.25		3	93	2	
0.50			6	91	2
2				7	98

[a] Underscored figures represent per cent correct from 300 samples at each concentration, or a total of 1500 samples.

be simple, specific, give a greater differentiation in the lower concentrations than Benedict's test, and be suitable for commercial production. There was developed from this work a test paper impregnated with the enzymes glucose oxidase, horseradish peroxidase, *o*-tolidine, and F.D.C. yellow No. 5 dye. This is now commercially available from Eli Lilly and Co. as Tes-Tape.

TEST PAPER

The test paper can be prepared in the laboratory by dipping analytical grade filter paper into 450 ml. of a 44% alcohol-water solution containing 1.9 grams of *o*-tolidine, 54,000 units of glucose oxidase, 34,000 P.Z. (purpurogallin zahl) units of horse-radish peroxidase, and 0.42 gram of F.D.C. yellow No. 5 dye, adjusted to pH 5.0 with formic acid.

The data presented here are from experiments performed with the commercially available paper prepared from carefully standardized enzymes by machine impregnation and drying. Comparisons were made with a color chart which comes with the paper and is graduated in yellow for negative glucose, light green for 0.10%, dark green for 0.25%, blue-green for 0.50%, and dark blue for 2% glucose. Minor changes are necessary in the color chart for each laboratory preparation of paper because of variations in the hand dipping process.

The paper is dipped into the urine specimen, removed, and, after 1 minute, the darkest area is compared to the color chart.

DISCUSSION

One of the first considerations of the possible sources of error for the semiquantitative use of the enzyme test paper was the influence of the pH variation of the urine. The buffering action of the paper was found to be sufficient, however, by conducting tests at pH values from 2 to 9 (in increments of 1 pH unit). At glucose concentrations of 0.10, 0.25, 0.50, and 2%, no differences were observed between the amount of glucose present and that found.

Temperature is known to alter the rate of enzymatic reaction, but there was no noticeable change in the results using the test paper under conditions of normal variation in room temperature. The temperature study was extended to specimen and room temperatures of 6°, 25°, 37°, and 50° C. for the 0.10 and 2% glucose concentrations, and there was still no variation from the color chart. This remarkable result may possibly be explained by the presence of another enzyme, catalase, which appears as a controlled impurity in the glucose oxidase.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Because the catalase is competitive with the peroxidase for the hydrogen peroxide, it may act as a governing agent on the test paper. Most of the variables such as pH, temperature, enzyme inhibitors, and accelerators, which change the rate of the reaction of oxidase and peroxidase, similarly change the rate of reaction of the catalase, resulting in only a small net change in the amount of peroxide available for the color reaction.

Several drugs were dissolved or suspended in urine containing 0.5% glucose. The urine was tested with the paper; no variation was observed from the 0.5% reading for glucose. The following drugs were used:

Acetophenetidin	Sulfathiazole
Acetylsalicylic acid	Theophylline
Amobarbital sodium	Thenylpyramine
Atropine sulfate	Tricyclamol sulfate
Caffeine	Reserpine
Cholesterol	Protoveratrine maleate
Diethylstilbestrol	Erythromycin
Ephedrine sulfate	Dihydrostreptomycin
Hyoscine hydrobromide	Penicillin potassium
Methyltestosterone	Vitamin A acetate
Morphine sulfate	Vitamin B_{12}
p-Aminosalicylic acid	α-Tocopherol
Procaine hydrochloride	Folic acid
Secobarbital sodium	Menadione
Sulfadiazine	Nicotinamide
Sulfamethazine	Nicotinic acid
Sulfamerazine	Pyridoxine hydrochloride
Sulfapyridine	Riboflavin

The concentration of the drugs was 10 mg. per ml. of urine, with the exception of reserpine, vitamin B_{12}, folic acid, and riboflavin, for which the concentration was 0.1 mg. per ml. of urine.

The screening of several thousand urine specimens from nondiabetic people has revealed no false positive reactions. To test further for false positive reactions, urine specimens were tested from both diabetic and nondiabetic patents under the various medications listed in Table I and no false positive reactions were observed with the test paper. Benedict's test was negative for all the patients with the exception of No. 14, which gave a trace reaction for glucose.

Urine solutions containing sucrose, galactose, lactose, xylose, and fructose gave no positive reaction. Ascorbic acid above 0.05% retards the color formation at the 0.10% glucose level. A false positive reaction is obtained with the Benedict's test with urine containing 0.05% ascorbic acid.

To ascertain the accuracy of the test paper, six panels of 10 people assayed a total of 1500 urine samples with a random distribution of 300 samples for each glucose concentration. The results of this study are shown in Table II; the over-all accuracy was 96%.

The data shown in Table III illustrate one of the basic differences in the test paper and the Benedict's test. Five people were allowed to familiarize themselves with the technique but not the color chart of both methods. They then tested 35 samples with random distribution of five samples for each of seven concentrations. The greater differentiation of the test paper for the 0.10, 0.25, and 0.50% glucose concentrations is evident, because most of the samples were called 1+ (approximately 0.25%) by the Benedict's method. The Benedict's test gave an accuracy of 72, 68, and 80% for the 1, 2, and 3% levels, respectively, indicated by the approximate 2+, 3+, and 4+ system of the Benedict's chart.

Table III. Comparison of Glucose Test Paper and Benedict's Test on Urine Solutions of Known Concentration

% Glucose Present	% Glucose Reported[a]	
	Tes-Tape	Benedict
0	24 = 0, 1 = 0.50	24 = neg., 1 = 1+
0.10	23 = 0.10, 1 = 0, 1 = 0.25	24 = 1+, 1 = 0
0.25	22 = 0.25, 1 = 0.10, 2 = 0.50	23 = 1+, 2 = 2+
0.50	21 = 0.50, 4 = 0.25	21 = 1+, 4 = 2+
1	14 = 0.50, 11 = 2	18 = 2+, 6 = 1+, 1 = 3+
2	22 = 2, 3 = 0.50	17 = 3+, 8 = 2+
3	25 = 2	20 = 4+, 5 = 3+

[a] Number of observations for five persons testing 35 samples with random distribution of five samples for each concentration.

Table IV. Comparison of Glucose Test Paper and Benedict's Test on Urine Specimens in Diabetic Clinic

Test Paper		Benedict's Test					
Glucose, %	No.	Neg.	Trace	+	++	+++	++++
0	240	234	6				
0.10	55	38	17				
0.25	45	3	39	3			
0.50	27		17	9			1
2	115		6	30	26	21	32

Urine specimens received at a diabetic clinic were tested with the Benedict's test and the test paper. Table IV lists the number of observations for the test paper and the corresponding observations for the Benedict's test. Again, better differentiation is evident by the test paper at the lower concentrations and by the Benedict's test for the range from 1 to 2% not covered by the color chart for the test paper. For better differentiation with the test paper at higher glucose concentrations, dilutions of the urine are necessary.

The test paper can be dipped conveniently into the specimens as they are received in the normal variety of containers. Four determinations can be made every 2 minutes by dipping one strip every 15 seconds during the first minute and reading one strip every 15 seconds during the second minute. In the timing interval the moist paper can be folded and hung from the edge of the specimen container or placed in order on adhesive cellophane tape.

The stability of the paper, as indicated by testing at intervals over a period of 6 months, was found to be satisfactory at room temperature, 37° C, and 50° C. when protected from light. Direct sunlight causes a gradual deterioration of the paper, but the opaque plastic dispenser, in which the commercially available paper is packaged, serves to protect it.

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RECEIVED for review March 14, 1956. Accepted August 3, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956.

Boron Hydride Monitoring Devices

Employing a Triphenyltetrazolium Chloride Reagent

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Two instruments may be used to monitor atmospheres contaminated with boron hydrides: a portable, field model with a hand-operated pump and a continuous analyzer which automatically records boron hydride concentration. Both were designed to make use of the nonspecific reduction of triphenyltetrazolium chloride by boron hydrides to form the red-colored formazan. Metered air samples are passed through filter paper or cloth tape impregnated with the reagent solution. The red color produced is measured by visual means with the field model and by a differential reflectance photometer with the automatic instrument. The instruments were calibrated with diborane, pentaborane, and decaborane; although they are relatively insensitive to diborane, very low concentrations of pentaborane and decaborane can be detected. The field model can detect 0.1 p.p.m. of decaborane and 0.5 p.p.m. of pentaborane, while the automatic instrument is capable of detecting 0.1 p.p.m. of either compound.

THE high toxicity of boron hydrides makes it desirable to have monitoring devices for the detection of very low concentrations of these compounds in areas where they may contaminate the atmosphere. The instruments described here are capable of detecting pentaborane and decaborane at least at the maximum allowable concentrations (MAC). For decaborane this concentration is less than 1 p.p.m. (*8*) and for pentaborane may be set at less than 0.2 p.p.m. (*6*).

It was first established by Hill that triphenyltetrazolium chloride (TTC) in alkaline solution is reduced by boron hydrides to form the red-colored formazan and it was employed by him in the quantitative estimation of these compounds (*3*). The color reaction is not specific for boranes; methods are reported in the literature for its use in the quantitative determination of sugars and of enzyme (dehydrogenase) activity (*5*, *7*). Although it is not specific, the high sensitivity of the reagent for these compounds justifies its use. A reagent developed in this laboratory is employed as the detecting element for the instruments described in this paper.

The reagent, which contains triphenyltetrazolium chloride, quinoline, pyridine, and water, is particularly suited for monitoring devices of this kind. It has a low volatility of the necessary alkalinity without being as unstable as an aqueous alkaline solution of the salt. The salt and its reduced form are both soluble in the solution, whereas the reduced form precipitates out of an aqueous solution.

A silver nitrate reagent is reported by Etherington and McCarty (*2*) for boron hydride detection with a monitoring device. Instruments similar to the automatic recording analyzer described here are available commercially for the detection of compounds other than boron hydrides (hydrogen sulfide analyzer, Rubicon Co., Philadelphia 32, Pa.; Microsensor, Vitro Corp., 233 Broadway, New York 7, N. Y.)

Some parts of the devices reported on in this paper had to be fabricated from stainless steel and Teflon, because of the action of the reagent on other metals, rubber, etc.

FIELD MODEL

The portable model consists of the sensing unit and the hand-operated vacuum pump (Figure 1). The stainless steel sensing

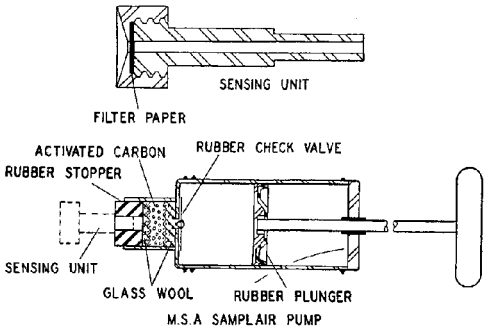

Figure 1. Hand-operated monitor for boron hydrides

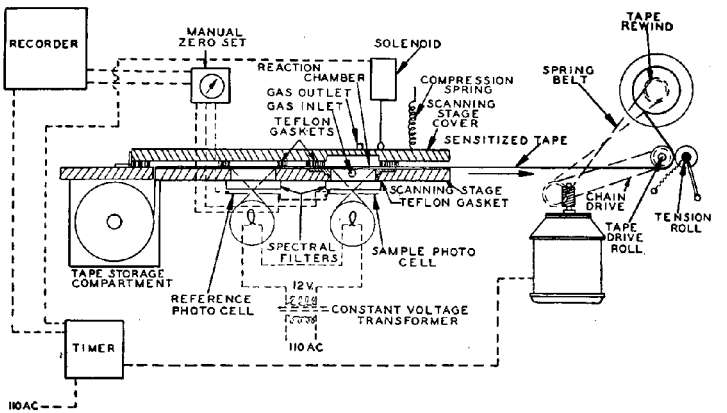

Figure 2. Schematic diagram of automatic recording monitor for boron hydrides

unit is designed to clamp the filter paper ($^3/_4$ inch in diameter) tightly in position and to allow air to be drawn only through an area of the paper $^1/_4$ inch in diameter. Observation of the paper was made easier by dishing the top of the cap.

The vacuum pump used for this device is part of the MSA Samplair, an instrument for detection of chromic acid mist. The sensing unit is inserted into a rubber stopper, which is in turn inserted into the neck of the pump. A layer of activated carbon placed between two layers of glass wool in the neck of the pump prevents the quinoline and pyridine in the reagent from attacking the rubber components of the pump.

In operation, a filter paper disk is placed in the sensing unit cap. Two drops of reagent are placed on the paper, and the cap is screwed on the body of the unit. Sample air is drawn through the reagent paper by means of the pump, and the paper is observed after each stroke. The number of strokes required to produce a definite, uniform pink color is recorded and compared with a calibration chart.

AUTOMATIC RECORDING MODEL

Description of Analyzer. Atmospheric boron hydride pollution is automatically measured and recorded by the automatic reflectometric analyzer, and it is possible to install easily an alarm circuit to be actuated at any boron hydride concentration within the instrument's range. A schematic drawing of the analyzer is shown in Figure 2.

When boron hydride–contaminated air is drawn through the tape impregnated with reagent, a red color is produced which changes the reflectance of the tape. A potential difference is obtained between the two photocells of the double-beam photometer and this potential is recorded. The rate of sample flow and exposure time are kept constant, and calibration data are obtained by relating recorder readings to the corresponding boron hydride concentrations.

The analyzer has the following operational sequence:

The tape is moved into position by the tape-pulling mechanism and clamped into place by the lowering of the scanning stage cover. During this operation, the sample flow is bypassed through a solenoid valve, and sample is not pulled through the tape until a 15-second zeroing time has elapsed. After the zero point is established, the sample is pulled through the tape for 3.5 minutes, and any deflection is recorded. The sequence is then repeated automatically.

Reflectance Photometer and Recorder. The reflectance photometer is composed of two Photovolt 610D search units with matching photocells. The original light filters are replaced with Corning 490-mμ glass filters (filter glass No. 4445 CS 4-74, $2^3/_{16} \pm {}^1/_{32}$ inch, polished stock thickness of 2.1 to 2.9 mm., edges ground standard quality). Unexposed tape is scanned by the reference photocell, and the exposed tape by the other. The two photocells are in a bridge-type circuit, and when no reductant is being sampled, a zero potential is obtained. A manual control is provided for setting this zero potential by balancing the output of the two photocells. The photometer lamps are fed from a constant-voltage transformer and are connected in series to eliminate the effect of current fluctuations.

The output of the photocells is fed into a Leeds & Northrup recorder with the range set at 0 to 10 mv.

Tape-Drive Mechanism. The tape-drive mechanism is controlled by the timer that controls the duration of the exposure. The sequence of the operation is initiated with the cover solenoid and the tape-drive motor being energized. The cover is lifted by the solenoid and the tape is drawn through the slot above the storage compartment, across the scanning stage, and through the drive and tension rolls and is then wound on the tape-rewind spool for easy disposal. The drive roll and rewind roll are turned by means of the same motor; a spring belt used on the motor allows for the increasing diameter of the tape-rewind roll. After the unexposed tape is positioned, the solenoid is de-energized and the cover is pulled into place by the two springs. At the end of the exposure period, the solenoid and motor are again energized, and the sequence is repeated.

Gas-Sampling System. Gas sample is drawn through the inlet, into the reaction chamber by way of an annular slot, and through the reagent tape. The spent sample then passes through a charcoal filter, and a vacuum pump which leads to a vent. Flow rate is regulated at 1 liter per minute by means of the needle valve and a Flowrator inserted in a sample line attached to the inlet. Solvent vapors and boron hydrides are removed by the charcoal filter to prevent them from going into the pump. The reaction chamber is sealed at the bottom by a Teflon gasket and the glass light filter, which are held in position by the photometer assembly and sealed at the top by a Teflon gasket seal between the scanning stage and the stage cover. The reagent tape is held in position by the latter seal, which also stretches the tape to give a uniform reflection. An absolutely tight seal is not obtained, because of the fibrous nature of the tape, but the leakage is not variable nor too great. Circular sections on the stage cover directly above the tape are painted white to provide a suitable reflectance background for the tape.

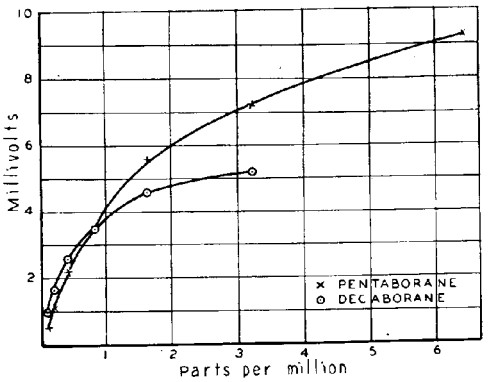

Figure 3. Calibration curves for automatic monitor

Sample flow through the reaction chamber is discontinued by means of a solenoid valve during the positioning of the tape and the 15-second zeroing time.

Timing Mechanism. For experimental purposes an automatic reset timer (Industrial Timer Corp., Model P-5M-Fig. 6) and a recycling timer (Industrial Timer Corp., Model MC3-814-4, cams, 46.5 seconds) were used. After the 3.5-minute exposure period, the solenoid valve is positioned so that sample is not pulled through the instrument. At the same time, the motor that pulls the tape and the solenoid that lifts the scanning stage cover are put into operation for 2 seconds. Fifteen seconds later the solenoid valve is positioned to allow sample air to flow through the instrument for the 3.5-minute exposure period.

EXPERIMENTAL

Diborane and pentaborane calibration samples (approximately 1000 p.p.m. in nitrogen) were contained in 4-liter steel cylinders at initial pressures of 1000 pounds per square inch gage. Lower concentrations were obtained by diluting with air using critical-orifice flowmeters (pressure drop greater than 1 atm.). These compounds were at least 98% pure, and as the concentrations in the cylinders were determined by analysis, no attempt at further purification was made. Diborane and pentaborane samples were determined as boric acid by the dianthrimide method (*1*). Decaborane (resublimed) samples were obtained by passing nitrogen through decaborane crystals held at 25° or 35° C. and diluting the nitrogen stream (not over 30 ml. per minute) with air, using critical-orifice flowmeters. Concentrations of decaborane were determined by Hill's quinoline method (*4*). Boron hydride samples for calibration were diluted at least 90% with air; erroneously high sensitivities were obtained when samples were diluted entirely with nitrogen.

Triphenyltetrazolium chloride was a product of Eastman Organic Chemicals, Rochester 3, N. Y., and the quinoline and pyridine were reagent grade and obtained from Fisher Scientific Co., Pittsburgh, Pa. The filter paper (grade FF 3, lot 3097) used in the field model is manufactured by the Hollingsworth and Vose Co., 41 Park Row, New York 7, N. Y.

Preparation of Reagent. The reagent is prepared by dissolving 0.5 gram of triphenyltetrazolium chloride (Eastman Organic Chemicals) in 2.5 ml. of pyridine (reagent grade) and 2.5 ml. of distilled water and then adding 25 ml. of quinoline (reagent grade) and shaking. This solution will keep indefinitely if stored in a dark bottle away from light.

Preparation of Reagent Tape. Reagent tape is prepared by placing one drop of reagent at 1/2-inch spacings along the tape. The tape is then rolled on a spool and allowed to stand overnight before using. It is recommended that the reagent tape be used within 1 week after preparation. The tape (Rubicon Co., Catalog No. 4805) is the same as in the hydrogen sulfide analyzer except for the absence of lead acetate reagent.

RESULTS AND DISCUSSION

The data obtained in the calibration of the instruments with diborane, pentaborane, and decaborane are given in Tables I and II. The instruments were so insensitive to diborane that complete calibrations were not made for this compound, but they can detect very low concentrations of pentaborane and decaborane and should be useful monitoring devices for these compounds. Calibration curves for pentaborane and decaborane obtained with the automatic analyzer are shown in Figure 3.

Table I. Calibrations for Field Model Borane Detector

No. of Strokes	Diborane, P.P.M.	Pentaborane, P.P.M.	Decaborane, P.P.M.
1	100	10.0	20
2	..	7.0	8
3	..	5.0	4
4	..	3.0	2
5	..	1.0	1
6	..	0.9	0.8
7	..	0.8	0.6
8	..	0.7	0.4
9	..	0.6	0.2
10	10	0.5	0.1

Table II. Calibrations for Automatic Reflectometric Borane Analyzer

P.P.M.	Av. Deflection, Mv.	No. of Analyses
	Diborane Calibration	
B_2H_6		
30	0.2	2
100	1.0	4
	Pentaborane Calibration	
B_5H_9		
6.4	9.3	3
3.2	7.2	4
1.6	5.6	8
0.8	3.5	3
0.4	2.3	4
0.2	1.1	4
0.1	0.5	8
	Decaborane Calibration	
$B_{10}H_{14}$		
3.2	5.2	4
1.6	4.6	3
0.8	3.5	3
0.4	2.6	5
0.2	1.7	4
0.1	1.0	6

It would be possible to increase the sensitivity of the field model somewhat by increasing the number of strokes and that of the automatic model by increasing either flow rate or exposure time. The amount of increase is limited by the fact that the reagent solvents gradually evaporate, making the reagent insensitive. New calibrations are required whenever operating conditions are changed, because any change greatly alters them. No long-term studies of reproducibility were made, but the small number of tests made show that the precision of the field model was about ±50% and of the automatic model within ±20% even at low concentrations.

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RECEIVED for review March 12, 1956. Accepted July 18, 1956.

Cyanogen-Oxygen Flame

New Source for Quantitative Determination of Microgram Amounts of Metals

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The cyanogen-oxygen flame has been successfully employed as a spectrochemical source for the quantitative determination of aluminum, barium, calcium, cobalt, chromium, copper, iron, lead, magnesium, manganese, nickel, and strontium. Absolute amounts varying from 0.36 to 36.0 γ of metals may be determined at the present time. When adequate quantities of the gas can be produced, the cyanogen-oxygen flame holds great promise as a refinement and simplification of trace metal analysis. The toxicity of cyanogen has not presented any problems during the experimental work.

THE flame, compared to the arc and spark, is a stable source. The intensity of radiation emitted is constant with time, and the spectra excited are relatively simple. For some elements, only two or three lines are observed, thus making it easy to isolate emission lines or band heads for measurement from the continuum. However, high resolution increases the line-background ratio, improving both the sensitivity and precision and minimizing interference from other radiating species in the flame (*7–9, 16*).

Table I. Temperatures of Some Flames

Fuel-Oxidant Mixture	Temperature, ° C. From Gaydon (*4*)	From Mavrodineau and Boiteux (*10*)
Hydrogen-air	2100	2115
Hydrogen-oxygen	2810	2690
Acetylene-air	2250	2050
Acetylene-oxygen	..	3110
Methane-air	..	1955
Methane-oxygen	2737	2720
Ethylene-air	..	1895
Propane-air	..	1925
Propane-oxygen	2776	..
Manufactured gas–air	..	1840
Manufactured gas–oxygen	..	2800
Cyanogen-oxygen	4580	..

The temperature of the flame is controlled largely by the energy of the reaction between fuel and oxidant, but it is markedly influenced by the further decomposition of the products of combustion and the heat capacity of the resultant fragments. Therefore, the temperature of the flame does not reflect all of the energy made available by the reaction. A portion of the initial energy is utilized to heat the fragments to the final flame temperature, which is thus a function of the fuel, oxidant, and sample employed (*4*). Table I lists the temperatures in degrees centigrade for various fuel-oxidant mixtures. The temperature of most flames conventionally used in analysis range from 2000° to 3100° K. (*4*). They are adequate only to excite elements of low excitation potential, such as the alkali metals and alkaline earths. These elements may be excited sufficiently so that high analytical sensitivity results, even though the flame is relatively cool. The cyanogen-oxygen (*13*) and hydrogen-fluorine flames (*11*), which have been the recent subject of physical studies, appeared to be potential sources for elements requiring higher excitation energies. Both of these flames have been shown to approach temperatures of 5000° K., considerably higher than temperatures currently obtainable with other flames. Such considerations led to an investigation of the usefulness of the cyanogen-oxygen flame as a spectroscopic source (*1, 14, 15*).

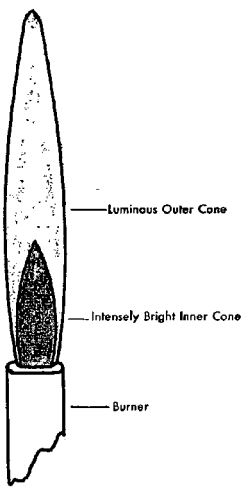

Figure 1. Schematic diagram of cyanogen-oxygen flame

The present paper presents the first quantitative spectrochemical data obtained with this source.

MATERIALS

Cyanogen was obtained from the Cyanamide Co. of America, New Products Division, New York, N. Y. A burner constructed in the authors' laboratory allows the combustion of cyanogen and oxygen and the introduction of the sample solution as a fine fog suspended in the oxygen stream. The oxygen is first passed through a nebulizer—an atomizer plus a trap—which allows for the return of large droplets. The sample fog and oxygen are premixed with equimolar amounts of cyanogen and flow out of the burner through a small hole, 1.1 mm. in diameter, at the top of the burner, where the flame is formed. The flame, diagrammatically shown in Figure 1, is narrow with a very bright, bluish-white inner cone and a blue outer cone. The flow rate of both cyanogen and oxygen is about 22 ml. per second.

The known toxicity of cyanogen occasioned precautions. Though they have not proved necessary thus far, such measures continue to be observed and are given here as background information.

Cyanogen has a characteristic, pungent odor, distinctly noticeable at concentrations far below its toxic levels in air (*6*), which is one of the best indications of leakage of the gas into the surrounding air space. When equimolar quantities of oxygen and

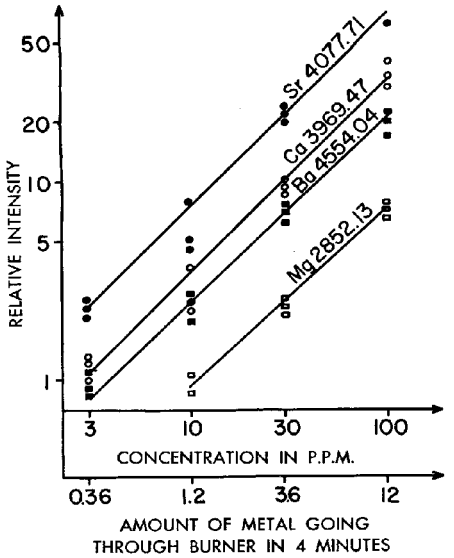

Figure 2. Working curves for strontium, calcium, barium, and magnesium

cyanogen interact, cyanogen cannot be detected among the resulting products, carbon monoxide and nitrogen. Because carbon monoxide, however, is odorless as well as toxic, it must be removed by exhaustion and adequate ventilation (1000 cubic feet per hour).

All rubber tubing was secured with copper wire, and the connections were wrapped with filter paper impregnated with a 3% ethyl alcohol solution of gum guaiac and a 0.1% solution of copper sulfate. When these reagents come in contact with cyanogen, the Schoenbein-Pagenstecher (*3*) reaction takes place instantly, resulting in an intense blue color which ultimately turns brownish red. In this manner slight leaks were detected instantly. Though never employed, a gas mask containing a canister for hydrogen cyanide gas (type GMK canister, Mine Safety Appliances Co., Pittsburgh, Pa.) was kept in readiness at all times.

The intense emission of ultraviolet light by this flame required the use of goggles to avoid burns.

Stock solutions (made with water as solvent) containing aluminum, barium, bismuth, cadmium, cobalt, chromium, copper, iron, lead, magnesium, manganese, molybdenum, nickel, tin, strontium, and zinc were made from Spec Pure metals or salts (Johnson Matthey Co., London, England) in concentrations of 10,000 p.p.m. Sample solutions of 0.33, 1.0, 3.3, 10, 30, 100, and 300 p.p.m. were prepared by serial transfers.

INSTRUMENTATION

A 1.5-meter Wadsworth spectrograph (Jarrell-Ash Co., Boston, Mass.), with a grating of 15,000 lines per inch, and Eastman Kodak type 103-0 film were employed for this work. The slit width was 65 microns. The reciprocal linear dispersion was 10.8 A. per mm. in the first order. The arrangements of the camera, in conjunction with the sensitivity of the film, confined selection of lines for measurement to the region between 2800 and 5200 A.

The exposure time was 4 minutes. The amount of sample consumed during this period was measured carefully to allow the calculation of the total amount of metal introduced into the burner per total exposure time. In this manner the absolute sensitivity of the method could be determined. A total of 0.12 cc. of solution passed through the burner during the 4 minutes' exposure time.

The film was developed for 5 minutes in D19 developer and fixed in a hypo bath for 20 minutes. No internal standard was employed. Densitometry was performed with an Applied Research Laboratories microdensitometer, the slit width of which was 10 microns.

Figure 2 shows working curves for the alkaline earth metals magnesium, calcium, strontium, and barium. Amounts of 3 p.p.m. of calcium, barium, and strontium and 10 p.p.m. of magnesium could be measured conveniently. The slopes of the curves are equal.

Figure 3 similarly represents the parallel working curves obtained with manganese lines 4034.490, 4033.073, and 4030.755, and Figure 4 shows working curves for the chromium lines 4234.05 and 4274.803 and the aluminum line 3961.52. The limits of detection are apparent from the figures.

The working curves for cobalt, iron, and nickel were similar to those shown in Figures 2 to 4.

All working curves are linear over a wide range and have slopes close to 45°. The sensitivity for molybdenum, tin, zinc, and cadmium was much poorer than that of the other elements analyzed. Unfortunately, the ultimate lines (raies ultimes) of these elements could not be measured because they lie outside of the range of the spectrograph which was employed.

DISCUSSION

The reactions of cyanogen with air and oxygen and their kinetics have been studied for many years. In 1914 Reis (*12*) analyzed the products and the spectrum of this flame. On the basis of theoretical considerations, he suggested the flame temperature to be 4740° C. Following renewed interest in this source, Thomas, Gaydon, and Brewer (*18*) measured the flame temperature experimentally by a determination of the vibrational intensity distribution of the cyanogen bands. They found it to be 4800° ± 200° K. in stoichiometric cyanogen-oxygen flames. Conway, Grosse, and Wilson (*2*) measured the temperature of the cyanogen flame directly by means of the line reversal method, using the sun as a comparison radiator, and found the temperature to be 4640° ± 50° K. when cyanogen and oxygen were equimolar. Their calculated value, 4835° ± 50° K. is in good agreement with that of Thomas, Gaydon, and Brewer. An increase in pressure to 10 atm. raised the flame temperature to 5050° K.

Calculation of the theoretical temperature was based upon the assumption that the reaction between cyanogen and oxygen is

$$C_2N_2 + O_2 \rightarrow 2CO + N_2 + E$$

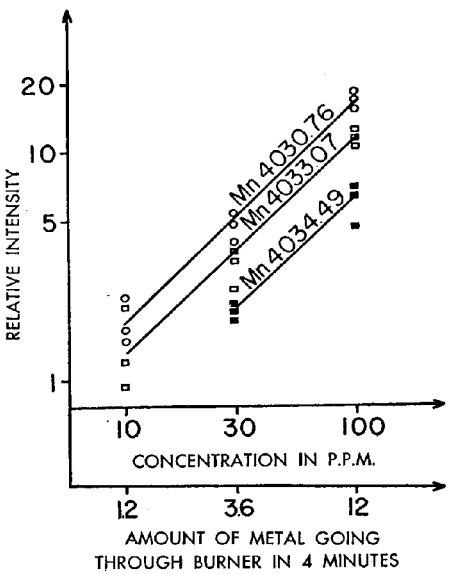

Figure 3. Working curves for three manganese lines

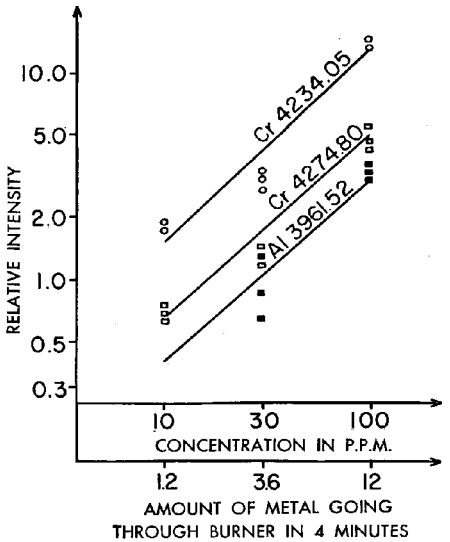

Figure 4. Working curves for two chromium lines and aluminum line

when equimolar quantities of both reactants are present. The high temperature of the flame is apparently due to the highly exothermic nature of the reaction, coupled with the fact that only a small fraction of its products dissociate. Theoretically, therefore, this flame should provide adequate energies to excite elements other than the alkali metals and the alkaline earths. The first data to be obtained on the excitation of spectra of elements in the cyanogen-oxygen flame have shown it to be a good spectroscopic source for the excitation of aluminum, barium, calcium, cobalt, chromium, copper, iron, magnesium, manganese, lead, and strontium (*1, 14, 15*). Interference in the spectra from the presence of cyanogen band heads, initially thought to be a major handicap, has been found to be of no consequence. The outer cone hardly emits cyanogen band spectra, and clear spectra devoid of cyanogen interference are readily observed. This is easily accomplished by proper alignment of the optics and the source in relation to the camera.

There are no data on the relative sensitivities to be expected for different lines of the same element in the cyanogen flame, of course. Figure 3 is particularly pertinent in this regard. In the "MIT Wavelength Tables," the manganese lines 4030.755, 4033.073, and 4034.490 are assigned relative arc intensities which compare as 5 to 4 to 2.5 (*5*). The working curves obtained for these lines intercept the log intensity axis at 1.8, 1.3, and 1.0 (Figure 3). The intensity ratios of these lines in the cyanogen-oxygen flame are 5 to 3.6 to 2.8, comparable to the intensities observed in the arc within the limits of experiment. Similarly, the "MIT Wavelength Tables" list arc intensity ratios for the three chromium lines, 4254.346, 4274.803, and 4289.721, as 5 to 4 to 3; in the cyanogen flame analogous results were obtained for these lines. The energy of excitation available in the cyanogen flame presumably causes electronic excitation phenomena closely akin to those occurring in the direct current arc.

The present data demonstrate the feasibility of quantitative spectrochemical analysis with this source. The data show good reproducibility on triplicate determinations, which is significant because an internal standard was not employed. The unit slope of the working curves is similar to that obtained for other sources under ideal conditions.

Because supplies of cyanogen were severely curtailed and were not sufficient for extended experimentation, the investigation was confined to examination of the most critical features of the flame. These circumstances precluded extensive investigation, modifications, and improvements in burner design, further adjustments of the flow rate of the gases, the optimal characteristics of the sample, and its optimal rate of introduction into the flame. Similarly, extensive studies of reproducibility and precision could not be undertaken. The choice of an internal standard was contingent upon detailed knowledge of the spectra of any elements to be employed for this purpose and the volatilization of their salts in this flame. The amounts of cyanogen available were insufficient to carry out such examination.

The data here presented show the analytical sensitivity obtainable with this flame in the quantitative identification of elements not generally subject to excitation with ordinary flames. The use of the hydrogen-oxygen flame under otherwise identical conditions did not result in any detectable spectra for the transition elements. Not only is the cyanogen-oxygen flame highly sensitive, but the quantitative measurements, exemplified by the working curves, are excellent in view of the fact that no internal standards could be employed. The limitations imposed by the presently restricted availability of the gas will constitute but a temporary delay in the implementation of the desirable features of this analytical procedure. Further improvement should result from the use of photomultiplier detectors in place of the photographic plates. The cyanogen-oxygen flame is a valuable addition to the sources presently available for spectrochemistry. Extension of this work should develop a useful technique in the analysis of many metals in the microgram and millimicrogram range.

ACKNOWLEDGMENT

The authors take pleasure in acknowledging the assistance and advice of Ralph E. Thiers.

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RECEIVED for review April 9, 1956. Accepted June 25, 1956. This work has been supported by a contract between the Office of Naval Research, Department of the Navy, and Harvard University, Contract NR 119-277, and by grants from the Howard Hughes Medical Institute and the Research Corp., New York, N. Y.

Microdetermination of Phosphorus

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The ascorbic acid method of Ammon and Hinsberg, modified by Lowry and associates, has been applied to the determination of phosphorus in whole blood, plasma, serum, and urine. A sensitivity about eight times that of the aminonaphtholsulfonic acid method permits the use of much smaller samples for measurement in conventional cells (as little as 0.15 γ of phosphorus can be determined in ordinary 3-ml. cuvettes.) A comparison with an accepted procedure on a number of samples showed that the ascorbic acid method gave essentially the same results.

A NEED has existed for a method for phosphorus sufficiently sensitive to dispense with microtechniques and special glassware and apparatus. This is true in cases where only small samples are available, or where a number of different analyses must be made on a limited amount of specimen. The procedure presented in this paper fills such a need.

Most sensitive methods for determining phosphorus in biological materials are based on the color formed by the reduction of a phosphomolybdate complex. Probably the most widely used method is that of Fiske and Subbarow (*2*), in which the reduction is carried out with sulfite and aminonaphtholsulfonic acid.

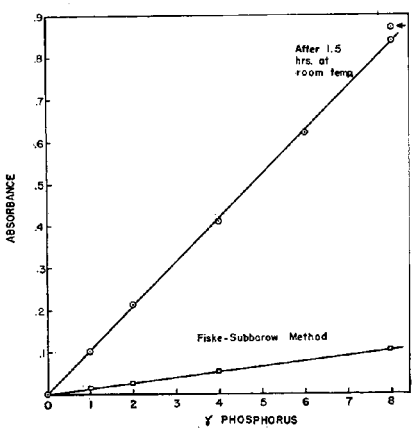

Figure 1. Standard curves

Upper. Ascorbic acid reduction
Lower. Sulfite–aminonaphtholsulfonic acid reduction

Ammon and Hinsberg (*1*) were the first to report the use of ascorbic acid for the reduction of phosphomolybdate, but it is difficult to calculate from their data the molar absorbance for comparison with other reports. Lowry and associates (*3*) modified the previous method by using a stronger ascorbic acid solution with a much longer time of heating at 37° C. and reported a molar absorbance of 25,000 as compared to 4000 for the Fiske and Subbarow method (*2*). The procedure of Lowry and associates (*3*) was developed for microgram quantities of brain and required a special adapter for the Beckman spectrophotometer to handle volumes of 40 μl. or less.

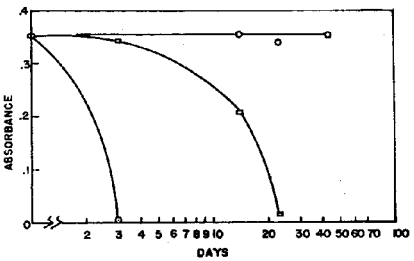

Figure 2. Stabilities of solutions

Colors developed from reagents standing for indicated number of days
Upper. Ascorbic acid kept at 4° C.
Middle. Reagent C kept at 4° C.
Lower. Reagent C kept at 25° C.

The present studies were designed to utilize the greater sensitivity of the ascorbic acid method for the determination of phosphorus in smaller quantities of whole blood, plasma, serum, and urine samples while using only conventional cells for the Beckman spectrophotometer or the Bausch & Lomb Spectronic 20 colorimeter. A thorough investigation has been made of the acidity, ascorbic acid concentration, stability of reagents under different conditions, and effect of time and temperature on the color. A comparison with the Fiske and Subbarow method has been made on a large number of serum and urine samples.

APPARATUS, REAGENTS, AND SOLUTIONS

Spectrophotometer. Beckman Model DU spectrophotometer with 1-cm. Corex cells. Used for comparison is a Bausch & Lomb Spectronic 20 colorimeter with 1P40 tube and red filter.

Reagent C. Mix 1 volume of 6*N* sulfuric acid with 2 volumes of distilled water and 1 volume of 2.5% ammonium molybdate, then add 1 volume of 10% ascorbic acid and mix well. Prepare fresh each day.

Ascorbic acid, 10%. Dissolve 10 grams of ascorbic acid USP (Mallinckrodt) in distilled water and dilute to 100 ml. Store under refrigeration at 2° to 4° C. The solution is stable for about 7 weeks.

Ammonium molybdate, 2.5%. Dissolve 2.5 grams of $(NH_4)_6Mo_7O_{24}.4H_2O$ (Baker's analyzed) in distilled water, and dilute to 100 ml.

Sulfuric acid (Baker's analyzed).

Sulfuric acid, 6*N*. Dilute 18 ml. of concentrated acid to 108 ml.

Perchloric acid (Baker's analyzed), 72%.

Hydrogen peroxide, 30% (Eimer & Amend tested purity).

Trichloroacetic acid, 10% (Baker's analyzed). Before using, check the phosphorus content; it is usually below detectable amounts when in the dilutions used for preparing filtrates, even though the stated analysis may indicate detectable quantities.

METHODS

Procedure. Pipet the phosphorus standards, diluted urine, ashed sample of biological material, or trichloroacetic acid fil-

trate (blood, plasma, or serum) containing up to 8 γ of phosphorus into a 15-ml. graduated centrifuge tube and adjust the volume to 4 ml. with distilled water. The reagent blank consists of 4 ml. of distilled water.

Pipet 4 ml. of reagent C into each tube, cap with Parafilm, mix, and place rack with all tubes in a 37° C. oven, incubator, or water bath for 1.5 to 2 hours. Remove, allow a few minutes to cool to room temperature, and read absorbance in Beckman DU spectrophotometer at 820 mμ against the blank.

Preparation of Samples. URINE PHOSPHORUS. Dilute urine with distilled water (if clear) or dilute hydrochloric acid (if turbid). If the approximate range is unknown, run several dilutions.

INORGANIC PHOSPHORUS. Add 0.5 ml. of whole blood, plasma, or serum to 2 ml. of 10% trichloroacetic acid. Mix well, centrifuge, and pipet off the filtrate. For routine analysis use 0.5 ml. of filtrate.

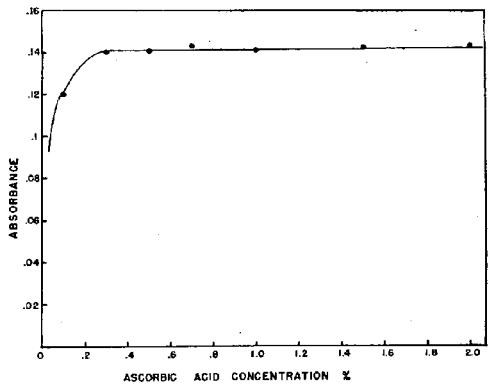

Figure 3. Effect of ascorbic acid concentration on 1.6 γ of phosphorus

LIPIDE PHOSPHORUS. Extract plasma, serum, or whole blood with alcohol-ether (3 to 1 by volume). Add 0.5 ml. of blood to 9.5 ml. of alcohol-ether and place in a hot water bath at 80° C. until boiling occurs. Remove, cool, and readjust to 10 ml. with alcohol-ether. Ash 5 ml. of filtrate or supernatant liquid as below.

Ashing Procedure. To determine total phosphorus first ash a sample of biological material. For lipide phosphorus, evaporate and ash the alcohol-ether extract. For total acid-soluble phosphorus of plasma, serum, or whole blood, ash trichloroacetic acid filtrates (see inorganic phosphorus above).

Place the sample to be ashed in a 20 × 150 mm. borosilicate glass test tube, add 4 drops of concentrated sulfuric acid (and 2.0 ml. of concentrated nitric acid if whole blood is to be ashed), and heat the test tubes over a sand bath until white fumes of sulfur trioxide appear. Then add 2 drops of 72% perchloric acid to each tube and heat in a Bunsen flame until the liquid becomes clear. After cooling, add distilled water and adjust the volume to 25 ml. in a volumetric flask. Aliquots can then be taken for analysis.

Concentrated (30%) hydrogen peroxide can be used instead of perchloric acid for the final ashing, provided it has been analyzed and found free from phosphorus. Many commercially obtainable C.P. peroxide solutions contain appreciable amounts of phosphorus. Ashing with peroxide is slower and all excess peroxide must be removed by boiling before phosphorus is analyzed.

STUDY OF VARIABLES

Selection of Wave Length. The absorption spectra of the colored phosphomolybdate reduction product was the same as that reported (*3*), and a wave length of 820 mμ was also used in this work. Absorption of the blank *vs.* distilled water is so low that for routine analysis a blank run is unnecessary.

Sensitivity of Method and Stability of Color. The sensitivity using ascorbic acid is about eight times that of the Fiske-Subbarow method, as is seen in Figure 1, which shows standard curves comparing the two procedures. Furthermore, the stability of the color development is very good. After 1.5 hours, the color intensity was increased only slightly.

Stability of Reagents. Reagent C is unstable and even if kept at 2° C. in the refrigerator will rapidly lose its ability to form a color with phosphorus. In Figure 2 are shown the relative stabilities of Reagent C and ascorbic acid solutions. The 10% ascorbic acid solution can be kept for many weeks at 2° C. in the refrigerator. The 6*N* sulfuric acid and 2.5% ammonium molybdate can be kept at room temperature.

Effect of Ascorbic Acid Concentration. As can be seen in Figure 3, the 1% ascorbic acid concentration used is well above that needed for maximum color development in the phosphorus range desired.

Effect of Time and Temperature. The color development at room temperature is incomplete even in 3 hours but is complete in 1 hour at 37° C. (see Figure 4). Ammon and Hinsberg found 7 minutes at 37° sufficient in their work, while Lowry and associates used 2 hours at 37° C.

Effect of Acid Concentration. In this method a rather broad but definite range of acid concentration is permissible. As is evident from Figure 5, blank solutions are reduced by ascorbic

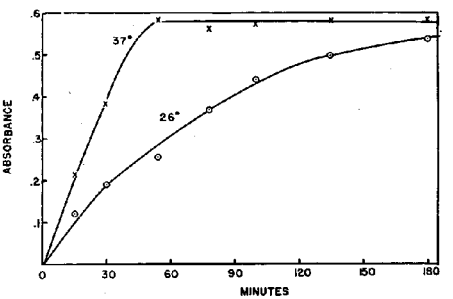

Figure 4. Effect of time and temperature on color development

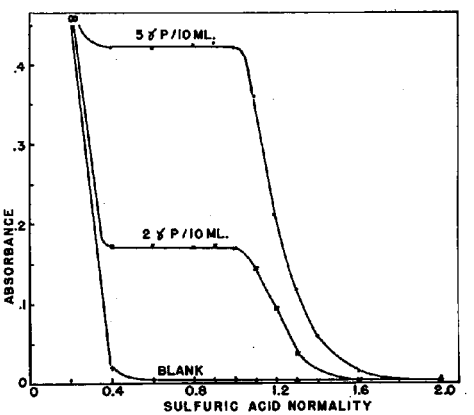

Figure 5. Effect of sulfuric acid concentration on color development at 37° C.

acid below an acid concentration of 0.4*N*. Above 1.0*N*, no reduction occurs even in the presence of phosphorus. Within the range 0.5 to 1.0*N*, color development is proportional to phosphorus concentration. The value of 0.6*N* was chosen to eliminate need for neutralizing the acids that may be present from the ashing procedure. The acidity of approximately 1*N* used by Ammon and Hinsberg and by Lowry and associates is at the upper limit.

Table I. Comparison between Ascorbic Acid and Aminonaphtholsulfonic Acid Reductions on Plasma

(Values indicate mg. of P per 100 ml. of plasma)

Ascorbic	ANSA	Ascorbic	ANSA	Ascorbic	ANSA
2.54	2.75	3.00	2.74	3.32	3.34
3.03	3.07	3.79	3.67	3.69	3.66
6.79	6.78	3.36	3.36	2.65	2.68
3.57	3.71	3.37	3.30	3.92	3.82
3.01	2.99	3.65	3.55	3.73	3.82
2.63	2.71	4.82	4.28	3.93	4.07
2.57	2.61	3.95	4.01	3.62	3.66
2.66	2.81	3.92	3.98	3.15	3.13
2.12	2.13	4.06	4.11	3.22	3.34
1.87	1.90	3.29	3.36	3.62	3.68

Av. diff. ± std. diff. = −0.006 ± 0.025.

Quantities of trichloroacetic acid up to four times that normally used for protein precipitation had no effect on the results. In the dilutions used such quantities would not appreciably change a 0.6*N* acid solution, so the results indicate no interference from the trichloroacetate ion.

COMPARISON WITH FISKE-SUBBAROW METHOD

A series of 30 blood plasmas and 20 diluted urines was analyzed for inorganic phosphorus by the Fiske-Subbarow method and by the suggested procedure. The plasma samples were obtained clinically and represent abnormal as well as normal values expressed as milligrams per 100 ml. in Table I. Urine values were obtained strictly for comparing the two methods and the dilutions were varied from 1:100 to 1:200 to bring the concentration of phosphorus within the proper range. The data were treated statistically and the application of the *t* test indicated that the methods gave identical results. The paired analyses of urine specimens showed an average difference of 0.03 ± 0.04 γ per ml.

TYPICAL BLOOD VALUES

A number of normal whole blood samples were analyzed by the procedures given for total blood, blood inorganic, blood acid-soluble, and blood lipide phosphorus. Typical values are listed in Table II.

DISCUSSION

The final concentration of 0.5 to 1.0*N* acid (exclusive of the 0.1*N* due to ascorbic acid) showed that the color development was proportional to phosphorus concentration. As reagent C, on final dilution, is 0.6*N* sulfuric acid, the sample itself may contain some acid, which may be trichloroacetic acid in plasma filtrates or sulfuric and perchloric acids from ashing.

In all routine analysis, no neutralization of trichloroacetic acid filtrates or acid from ashing is necessary. For example, the addition of 0.5 ml. of trichloroacetic acid filtrate in an 8-ml. final volume would give only 0.03*N* acid. In ashing, usually less than 0.5 ml. of concentrated sulfuric acid and perchloric acid is used. After dilution, aliquots containing 0.05 ml. or less are contained in the final tube, which is much less than the 0.4*N* safety factor allowed. 3.2 meq. of acid = 0.088 ml. of concentrated sulfuric acid in 8-ml. final volume.

The presence of arsenate interferes with phosphorus determination, as it also forms a colored reduction product (*1*) with molybdate. Eight micrograms of arsenic are equivalent to 1 γ of phosphorus, and the absorbance is proportional to its concentration. Silicate in amounts up to 1 mg. has no effect on phosphorus determination.

Although the method as described is usable with 0.5 γ of phosphorus in 4 ml. or 0.1 γ per ml., by slight modification it is adaptable to smaller total amounts. By using 1.5 ml. of trichloroacetic acid filtrate and 1.5 ml. of reagent C, 0.15 γ of total phosphorus can be accurately determined without the use of microcuvettes.

A wave length of 820 mμ precludes the use of most colorimeters. The Bausch & Lomb Spectronic 20 colorimeter with 1P40 phototube and red filter may be used in this range and was compared with the Beckman DU instrument. An absorption maximum at 820 mμ was obtained and standard curves were linear as in the Beckman. However, absorbance was higher for a given solution in the Spectronic. Thus 8 γ of P ≅ 1.0 (Spectronic) *vs.* 0.850 (Beckman).

Besides filling the need for a more sensitive phosphorus micromethod not requiring special microtechniques, this procedure also possesses the advantages of stability, constancy, and linearity.

Table II. Normal Values for Whole Blood Phosphorus Determined by Ascorbic Acid Method

Mg. of P per 100 Ml. of Blood			
Inorganic	Acid-soluble	Lipide	Total
3.71	26.3	10.5	31.0
3.21	23.7	11.3	31.0
2.48	21.9	11.4	31.6
2.96	23.3	12.7	36.8
3.21	23.6	10.9	31.1
3.30	22.2	10.2	35.0
2.84	22.5		32.7
3.32	25.8		33.0
			34.6

Stability of the color developed eliminated the necessity of reading solutions immediately or at a certain time after starting a color reaction. Constancy meant that with different batches of ascorbic acid and ammonium molybdate, 8 γ of phosphorus always read 0.840 to 0.850 on several Beckman DU spectrophotometers. Standard curves were always linear over the entire range of the Beckman instrument. For ordinary analysis requiring an accuracy within ±2%, blanks and standards need not be run each time. In the Fiske and Subbarow procedure (*2*) the absorbance of the blank *vs.* distilled water as well as the slope of the standard curve may vary from day to day.

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RECEIVED for review April 26, 1956. Accepted July 12, 1956. Based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.

Determination of Calcium and Magnesium in Foodstuffs

Simultaneous Removal of Iron and Phosphate as Interfering Ions by Ion Exchange

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A titration method using ethylenediamine tetraacetate is described for the routine determination of calcium and magnesium in foodstuffs such as bread, enriching mixtures, and grains, in which iron and phosphate occur in interfering amounts. Murexide and Eriochrome Black T are used as indicators for calcium and magnesium, respectively. The iron is converted into an oxalato complex and is removed with the phosphate by means of a cation exchange resin in the hydrogen form in a small column. The calcium and magnesium are eluted with hydrochloric acid and determined in the eluate; the end points are completely satisfactory. The method is simple and much less time-consuming than the oxalate and oxinate methods, and results compare favorably with those obtained by the classical methods.

THE determination of calcium and magnesium by disodium ethylenediamine tetraacetate [(ethylene dinitrilo)tetraacetate, EDTA] provides a rapid and simple method for routine analysis. This method, however, is subject to interference from many ions (*4*), and the elimination of ions present in interfering amounts becomes a necessity for efficient determinations.

The foodstuffs analyzed contained mainly orthophosphate and iron in varying amounts with traces of copper, cobalt, zinc, and manganese as interfering ions.

The interference from copper, cobalt, and zinc can be eliminated effectively by the addition of potassium cyanide (*1, 3, 4*). The manganese remains in solution and is titrated with the EDTA (*1*) but the error is negligible (*9*).

Methods for the separation of orthophosphate by means of ion exchangers have been described (*2, 6–12*). In the present investigation Amberlite IR-112, a medium capacity cation exchange resin of the sulfonic acid type, proved successful in the acid range investigated—pH 2 to 5.

The removal of iron was effected simultaneously with the phosphate by converting the iron into an anion complex. Although the elimination of the interference from iron using potassium cyanide and hydroxylamine hydrochloride has been described (*1, 4*), this method was found to be unsatisfactory, owing to an undesirable color which formed in the solution. The color of the indicator could hardly be discerned, making the determination of the end point practically impossible. The possibility of achieving a quantitative separation of iron from the other metals, among them calcium and magnesium, by percolation of oxalate solutions of the metals through cation exchangers, has been demonstrated by Djurfeldt, Hansen, and Samuelson (*5*). Following this line of investigation, experiments were carried out with artificial solutions containing calcium and varying amounts of iron.

A number of solutions were made up containing 8 mg. of calcium and from 4 to 10 mg. of iron per portion. Each portion was boiled with 1 ml. of hydrogen peroxide to ensure the oxidation of all the iron. Volumes of 5, 10, and 15 ml. of a saturated oxalic acid solution were added to each combination of calcium and iron, and then boiled for a few minutes. After cooling, the solutions were percolated through the cation exchanger, and the resin columns were washed with 0.2*M* oxalic acid, followed by 0.015*N* hydrochloric acid to displace the oxalic acid. The columns were eluted with 4*N* hydrochloric acid, followed by water. This eluate was evaporated to dryness to remove excess hydrochloric acid, the residue was dissolved in water, and the calcium was determined with EDTA, Murexide being used as indicator.

The results showed that where 5- and 10-ml. portions of the saturated oxalic acid solution were added, insufficient complexing agent was present to remove the iron quantitatively even in the smallest amounts present and no satisfactory end point could be obtained. With the 15-ml. portions, however, a practically quantitative separation was effected in all cases and the end point was satisfactory, complete recovery of the calcium being obtained, as can be seen from Table I.

No definite dimensions could be laid down for the resin column, as varying amounts of cations accompanied an amount of calcium giving a convenient titer. For rapid determinations the column must be as small as possible, however, within certain limits of safety that ensure the quantitative adsorption of calcium and magnesium. A number of columns with resin beds 0.8 cm. in diameter and 10 cm. in height were constructed. Since smaller particle size increases the breakthrough capacity of a resin column, the flow rate was adjusted to approximately 2 to 3 ml. per sq. cm. per minute by grinding the commercially available resin and using the fraction that passed through the 50-mesh (U. S. number) sieve but was retained by the 120-mesh sieve. Recovery experiments revealed that up to 4 meq. of cations could be percolated through the columns at a flow rate of 2 to 3 ml. per sq. cm. per minute and be adsorbed quantitatively (Table II). This amount was considered satisfactory for the conditions of use.

Table I. Recovery of Calcium after Quantitative Removal of Iron

Present, Mg.		Ca Determined, Mg.
Fe	Ca	
4	8.00	8.02
6	8.00	8.01
8	8.00	7.98
10	8.00	7.98

Table II. Range of Quantitative Recovery of Calcium Percolated through Resin Column

Percolated through Column, Mg.	Recovered, Mg.
5.00	4.95, 5.00
10.00	10.00, 10.00
20.00	20.00, 19.97
30.00	30.03, 30.01
40.00	39.94, 39.98
60.00	60.05, 60.01
80.00	79.98, 80.05
100.00	94.50, 93.97

The above quantities and dimensions were found to be satisfactory in the case of the resin used—Amberlite IR-112—and the particular particle size mentioned. When another resin or particle

size is used, it may be necessary to adjust the volumes of oxalic acid and 0.015N hydrochloric stipulated in the procedure given below.

Analyses on the foostuffs were also carried out by the oxalate and oxinate methods. The results obtained by these methods are compared with those by the direct titration method with EDTA in Tables III, IV, and V.

Table III. Calcium and Magnesium Content of Different Materials

(Determined by gravimetric methods against volumetric method using EDTA as titrating agent. Tabulated results are mean of a number o determinations.)

Material	Calcium Content/Sample, Mg.			Magnesium Content/-Sample, Mg.		
	Oxalate	EDTA	Difference	Oxinate	EDTA	Difference
Bread 8	5.30	5.35	+0.05	2.26	2.31	+0.05
Bread 10	5.92	5.99	+0.07	2.27	2.30	+0.03
Enriching mixture	4.11	4.03	−0.08	0.26	0.24	−0.02
Soybean meal	3.57	3.58	+0.01	2.52	2.45	−0.07
Corn meal	0.48	0.48	0.00	3.40	3.34	−0.06

Table IV. Standard Deviation from Mean Value

Material	Calcium, Mg./Sample		Magnesium, Mg./Sample	
	Oxalate	EDTA	Oxinate	EDTA
Bread 8	0.070	0.010	0.066	0.008
Bread 10	0.085	0.008	0.052	0.022
Enriching mixture	0.011	0.009	0.025	0.002
Soybean meal	0.019	0.014	0.082	0.001
Corn meal	0.026	0.016	0.070	0.010

Table V. Coefficient of Variation (Per Cent)

Material	Calcium		Magnesium	
	Oxalate	EDTA	Oxinate	EDTA
Bread 8	1.32	0.19	2.92	0.35
Bread 10	1.44	0.13	2.29	0.96
Enriching mixture	0.27	0.22	9.62	0.83
Soybean meal	0.53	0.39	3.26	0.41
Corn meal	5.42	3.34	2.03	0.30

PROCEDURE FOR ROUTINE ANALYSIS

Reagents. EDTA Solution. Dissolve 4 grams of disodium ethylenediamine tetraacetate in water, add 0.2 gram of magnesium chloride crystals, and dilute to 1 liter. Allow to stand overnight and then filter the solution.

Standard Calcium Chloride Solution. Dissolve 1.0000 gram of calcium carbonate, previously dried for 3 hours at 105° C., in the minimum quantity of dilute hydrochloric acid, transfer to a standard 1-liter flask, and fill up to the mark with water.

Murexide Indicator. Grind together in a mortar and mix thoroughly 0.1 gram of ammonium purpurate and 100 grams of dry analytical grade sodium chloride.

Eriochrome Black T Indicator. Dissolve 0.5 gram of Eriochrome Black T-241 with 4.5 grams of hydroxylamine hydrochloride in 100 ml. of ethyl alcohol.

Sodium hydroxide solution, 4N.

Ammonia–Ammonium Chloride Buffer. Add 67.5 grams of ammonium chloride to 570 ml. of concentrated ammonium hydroxide and dilute to 1 liter.

Hydrochloric acid, 4N and 0.015N.

Oxalic acid, saturated and 0.2M.

Hydrogen peroxide, 30 per cent by weight.

Potassium cyanide, analytical grade.

Hydroxylamine hydrochloride, analytical grade.

Amberlite IR-112 Resin. Prepare according to the usual method (*10*), using the fraction passing through the 50-mesh (U. S. number) sieve but retained by the 120-mesh sieve.

Apparatus. Resin columns, of standard type (*10*).

Standardization of EDTA solution. With Murexide. Transfer by means of a pipet 10 ml. of the standard calcium chloride solution into a conical flask of 250-ml. capacity. Add 1 ml. of sodium hydroxide solution and approximately 0.15 gram of Murexide indicator, the latter from a small measure made to this capacity. Titrate with EDTA solution until the color changes from pink to a definite mauve, using a blank for comparison. Repeat until duplicate values are obtained.

Calculate x, where

$$1 \text{ ml. of EDTA solution} = x \text{ equivalents}$$

With Eriochrome Black T. Transfer by means of a pipet 10 ml. of the standard calcium chloride solution into a conical flask of 250-ml. capacity. Add 1 ml. of the ammonia–ammonium chloride buffer and 6 drops of Eriochrome Black T indicator. Titrate with EDTA solution until the color changes to a clear blue. Repeat until duplicate values are obtained.

Calculate y, where

$$1 \text{ ml. of EDTA solution} = y \text{ equivalents}$$

METHOD

Dissolve the ash of a sample that will give a convenient titer and evaporate to dryness with hydrochloric acid, in 25 to 30 ml. of water and heat on the steam bath until the volume is 15 to 20 ml.

Procedure. For materials containing iron with or without orthophosphate in interfering amounts:

Transfer the solution of the salts obtained above into a 150-ml. flask. Add 1 ml. of hydrogen peroxide, cover with a watch glass, and heat on a hot plate. When the solution boils, add 15 ml. of saturated oxalic acid solution and heat for an additional 5 minutes. Cool and transfer to the cation exchange column. Wash the column with two separate 15-ml. portions of 0.2M oxalic acid solution followed by two 10-ml. portions of 0.015N hydrochloric acid. Reject the effluents. Place a 250-ml. conical flask under the column and elute the calcium and magnesium with one 15-ml. portion of 4N hydrochloric acid, followed by 20 ml. of water. When approximately half of the water portion has passed through the column, place the flask on a hot plate and evaporate to dryness. Dissolve the residue in approximately 50 ml. of water.

Determination of Calcium. Add 0.2 gram of hydroxylamine hydrochloride to the solution, shake to dissolve, and then add 0.25 gram of potassium cyanide. Add 1 ml. of sodium hydroxide solution and approximately 0.15 gram of Murexide indicator. Titrate with EDTA solution until the color changes from pink to a definite mauve, using a blank for comparison. Carry out the titration preferably against a background of artificial light (fluorescent light).

$$Ca^{++} \text{ (mg. per 100 grams)} = \frac{xn \times 20.04 \times 1000 \times 100}{M}$$

where x = number of equivalents per ml. of EDTA solution
n = titer (ml.)
M = weight of sample

Determination of Magnesium. Add 0.2 gram of hydroxylamine hydrochloride to the solution, shake to dissolve, and then add 0.25 gram of potassium cyanide. Add 1 ml. of ammonia–ammonium chloride buffer and 6 drops of Eriochrome Black T indicator. Titrate with EDTA solution until the color changes to a clear blue.

$$Mg^{++} \text{ (mg. per 100 grams)} = \frac{(ym - xn)\ 12.16 \times 1000 \times 100}{M}$$

where y = number of equivalents per ml. of EDTA solution (Eriochrome Black T as indicator)
m = $Ca^{++} + Mg^{++}$ titer, ml.
x = number of equivalents per ml. of EDTA solution (Murexide as indicator)
n = Ca^{++} titer, ml.
M = weight of sample

ACKNOWLEDGMENT

G. N. Krynauw received financial assistance from the South African Nutritional Research Institute of the Council for Scientific and Industrial Research, Pretoria and Agricura Laboratories, Ltd., Silverton, Transvaal.

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RECEIVED for review December 7, 1955. Accepted June 18, 1956.

Determination of Traces of Mercury in Mercury Ore Ash by Catalytic Action of Mercuric Ions

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The determination of traces of mercury in burnt mercury ore was carried out on the basis of the catalytic action of mercuric ions on the reaction between potassium ferrocyanide and nitrosobenzene, in which a violet complex $[Fe(CN)_5(C_6H_5NO)]^{---}$ is formed. In the range where the mercury in the ash amounted to 0.0024 to 0.0097% the relative error of the analyses varied between 6 and 2%, and the standard deviation was approximately 0.00015.

THE determination of small amounts of mercury in burnt ore that remains in mercury mines serves as a control of the smelting process in industry. The well burnt ore usually contains only a few thousandths of 1% of mercury. Such small amounts of mercury cannot be accurately determined by the classical Eschka method (*3*), which is accurate only when the quantity of mercury in the ore is greater than 0.1%. On the other hand, the temperature used in the Eschka method to expel mercury from the ash proved to be insufficient because of the adsorption of mercury on the ash. It appears that the other more sensitive methods for determination of mercury have not been applied to this case.

Proceeding with earlier experiments on the determination of small amounts of mercury (*1*), the authors used the catalytic action of mercuric ions on the reaction of potassium ferrocyanide and nitrosobenzene in aqueous solutions to determine traces of mercury in mercury ore residue. The reaction proceeds according to Equations 1, 2, and 3 (*2*):

$$Fe(CN)_6^{----} \overset{H_2O}{\rightleftarrows} [Fe(CN)_5(H_2O)]^{---} + CN^- \quad (1)$$

$$[Fe(CN)_5(H_2O)]^{---} + C_6H_5NO \rightarrow \underset{\text{violet}}{[Fe(CN)_5(C_6H_5NO)]^{---}} + H_2O \quad (2)$$

$$CN^- + H_2O \rightleftarrows HCN + OH^- \quad (3)$$

The concentration of the violet reaction product $[Fe(CN)_5(C_6H_5NO)]^{---}$ at a fixed reaction time depends on the concentration of mercuric ions present in the solution. By measuring the absorbance of the violet complex at 528 mμ, as little as 2 γ of mercury could be determined.

The ore residue was heated to 1200° C. and the mercury vapors liberated were mixed with bromine vapors according to the procedure of Moldawskij (*4, 5*), and absorbed in bromine water, and the mercuric bromide was determined by an earlier procedure (*1*).

EXPERIMENTAL

The ash (0.2 to 0.6 gram) was heated for 1.5 hours at 1200° C. and a stream of fresh air was passed through the furnace. The apparatus for oxidizing and absorbing mercury vapors with bromine vapors (*4, 5*) was attached to the furnace. After the liberation of mercury was completed, the glass tubes that connected the furnace and the absorption vessel were rinsed with bromine water, which was added to the bromine water of the absorption cell. The absorption solution containing traces of mercuric ions was then treated for the determination of traces of mercury in the atmosphere (*1*). The concentration of the mercuric ions was calculated from the equation

$$\log_{10}C_{Hg^{++}} = 1.112 \log_{10}E_{30} - 4.427 \quad (4)$$

where E_{30} is the absorbance of the violet reaction product at 30 minutes at 528 mμ and a path length of 1 cm. (*1*).

The table shows the results of the analysis of a sample under different conditions of burning.

Temp., ° C.	Time of Heating, Min.	Mercury Found in Ash, %
900	10	0.0025
	15	0.0029
	60	0.0031
	90	0.0035
1000	90	0.0041
	180	0.0043
1050	240	0.0044
1100	90	0.0051
1200	90	0.0051

Increase of temperature above 1200° C. and prolonged heating (over 90 minutes) caused an increase of the acidity of the otherwise slightly acid absorption solution to pH 2 or less. This is not desirable, because more sodium hydroxide would be needed for the subsequent adjustment of the pH to 3.5 before addition of potassium ferrocyanide, as required by the earlier procedure (*1*). This would cause an increase of the otherwise negligible negative salt effect on the catalyzed reaction, and hence slightly lower results in the analyses. The reaction shows a negative salt effect, as the charges of ferrocyanide and mercury ions are opposite in sign.

Fifty analyses on ash from the Idria mercury mine in Yugoslavia were carried out on samples containing 0.0024 to 0.0097% of mercury. The standard deviation was approximately 0.00015 in this whole range and the relative error varied between approximately 6% for the ash of low mercury content and 2% for the ash of higher mercury content.

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RECEIVED for review June 2, 1956. Accepted June 16, 1956.

Rapid Determination of Radiocarbon in Animal Tissues

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A simple technique was developed for solid counting of radiocarbon samples prepared from animal tissues. The dried tissues and organs containing cortisol-4-C^{14} were dissolved in hot formamide, and aliquots of the solutions were dried on aluminum planchets. The self-absorption of these cortisol-4-C^{14} muscle preparations was found to be linearly proportional to the sample thickness. Use of lens paper improved the consistency of the counts. The method is considered well suited for a comparatively rapid determination of radioactivity in animal material.

ORGANIC compounds labeled with radiocarbon have become important tools in studies on the distribution and metabolism of these substances in the living organism. In the determination of carbon-14 containing material, highest accuracy is generally obtained by counting carbon-14 as carbon dioxide or as barium carbonate (*1*). On this basis, Skipper, Bryan, White, and Hutchison (*10*) have described techniques for combustion of animal tissues for carbon-14 assay. Riegel, Hartop, and Kittinger (*8*) supplemented these methods by the use of extraction and saponification procedures. The radioactive carbon injected was accounted for with an over-all accuracy within ±10% (*10*).

To survey the distribution of a carbon-14–tagged compound in a great variety of organs and in a large number of experimental animals, any procedure involving such steps as combustion, solvent extraction, or saponification is very time-consuming. Therefore, a simple technique for direct measurement of the radioactivity in tissues is needed. The present report describes a solid-counting method that was developed for a study of the distribution of cortisol-4-C^{14} in the white rat. This procedure makes use of the finding by Tabern and Lahr (*12*) that whole organs or tissues form clear colloidal solutions in formamide.

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EXPERIMENTAL

Dissolution of Tissues in Formamide. The various organs and tissues were removed from the experimental animal (rat, injected with cortisol-4-C^{14}), cut into small pieces, and dried to constant weight (2 or 3 days) in a ventilated oven at 80° C. The dry tissues were placed in beakers and covered completely with formamide (Fisher reagent grade, or Matheson technical grade). The mixture was heated on a hot plate at about 105° C. for half an hour and the dissolving of the tissue was completed by raising the temperature to about 210° C. during an additional hour (hood). The mixture was stirred throughout these operations. Fresh formamide was added to replace loss by evaporation. The solution was brought to the desired volume with 95% ethyl alcohol. This procedure also dissolves skin and hair, but not bone.

The solutions obtained were homogeneous and in general clear. Liver and gastrointestinal tract sometimes did not dissolve entirely; in this case the insoluble material was removed and extracted thoroughly with additional formamide and 95% ethyl alcohol. The extracts were added to the original solutions; the residue was found to be free of radioactivity and, therefore, was discarded. The color of the colloidal solutions varied from light to dark brown. It was found essential for rapid dissolution to have the tissue in a completely dry state. Separate experiments showed that heating the radioactive material (cortisol-4-C^{14}) in formamide at 210° C. for 60 to 90 minutes and drying under an infrared lamp did not cause any loss of radioactivity. Attempts to dissolve the animal tissues in dimethylformamide were unsuccessful.

Determination of Radioactivity. The solutions were plated on aluminum planchets which were 30 mm. in diameter and had a 2-mm. rim. To obtain a uniform radioactive layer, the planchets were prewashed with petroleum ether or ether, acetone, a mild detergent solution, for 45 minutes at about 40° C., and finally with distilled water; the planchets then were dried. Care must be taken during these operations that the planchets do not stick together. Satisfactory washing was accomplished when a drop of

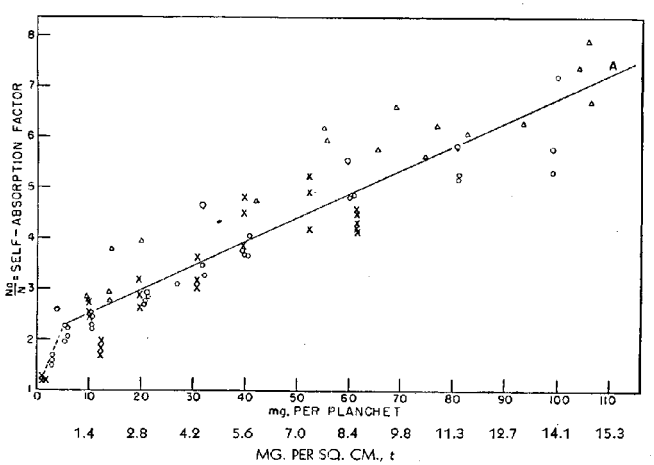

Figure 1. Self-absorption of cortisol-4-C^{14}-muscle preparations without lens paper

Levels of constant activity:
× 3000 c.p.m.
△ 400 c.p.m.
○ 40 c.p.m.
A. $No/N = 2.04 + 0.34\,t$

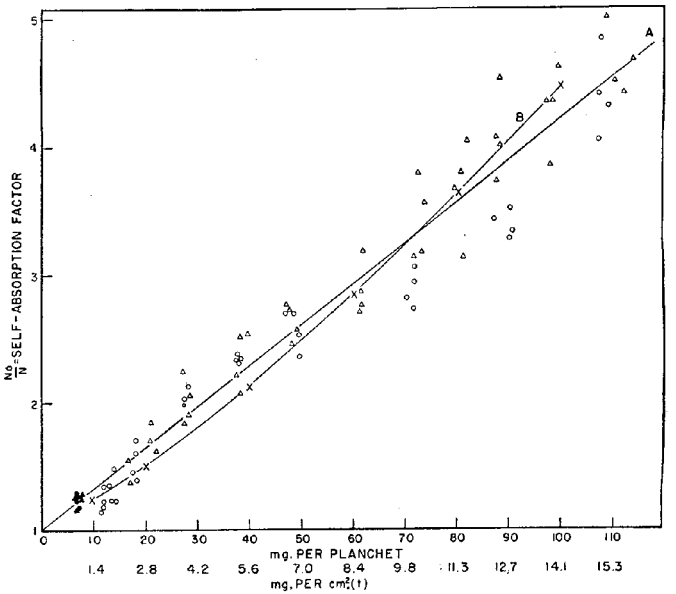

Figure 2. Self-absorption of cortisol-4-C^{14}-muscle preparations with lens paper

Levels of constant activity
△ 400 c.p.m.
○ 40 c.p.m.
●, ▲ Values obtained with weightless cortisol-4-C^{14} on lens paper
A. $No/N = 1 + 0.23\ t$
B. ×. $No/N = 0.31\ t/(1 - e^{-0.31t})$

distilled water dispersed uniformly over the whole surface of the dried planchet. A dilute solution of silicone stopcock grease in chloroform was applied to the inner surface of the rim and in a ring 1 to 2 mm. wide to the adjacent horizontal area. A thin film of silicone remained on this surface and helped to prevent the sample from spreading beyond the silicone-free center area. Accumulation of the material around the rim and creeping over it thus was avoided. The consistency of counts obtained in quadruplicate determinations was increased considerably by the use of lens paper (*1, 4*). Circles 29 mm. in diameter were cut out with the aid of a steel punch and placed on the planchets before the solution was applied. As the average weight of a lens paper circle was found to be 7 mg., their thickness was approximately 1 mg. per sq. cm.

The volumes of the tissue-formamide solutions were adjusted so that the material to be counted was contained in 0.5 ml. The solution was pipetted onto the planchet and dried for approximately 12 hours under an infrared lamp. The safety pipetting device used has been described (*14*). The dried samples were stored in a desiccator. The radioactivity was measured with a Q-gas flow counter (Model D46, Nuclear Instrument and Chemical Corp., Chicago 10, Ill.) or in an automatic gas flow counter (Model SC-50, Tracerlab, Inc., Boston 10, Mass.). The counts per minute (c.p.m.) were corrected for background and for small daily variations in the response of the counters by referring to a standard polystyrene source labeled with carbon-14 which was obtained from Oak Ridge National Laboratory on allocation from the Isotope Division, U. S. Atomic Energy Commission.

Self-Absorption Curves. Self-absorption curves of constant activity were prepared using a muscle-formamide–ethyl alcohol stock solution, the dry weight of which was determined after drying as described above. A constant amount of cortisol-4-C^{14} [specific activity 4.08 μc. per mg.; radiochemical purity higher than 94% (*13*)] was added to varying quantities of the dissolved muscle material. The cortisol-4-C^{14} solution, the muscle-formamide solution, and 95% ethyl alcohol were mixed in such proportions that the volume of the final solution plated (0.5 ml.) contained between 5 and 100 mg. of dry tissue. The amount of cortisol-4-C^{14} in these mixtures was adjusted so that three levels of radioactivity were obtained—i.e., approximately 3000, 400, and 40 counts per minute per 0.5 ml. The planchets were prepared in triplicate or quadruplicate without lens paper (Figure 1) and, at the two lower levels of radioactivity, with lens paper (Figure 2). The reference values ("100%") for the radioactivity in the experiments without lens paper were determined on a total of 20 planchets with essentially weightless samples (less than 0.14 γ of cortisol-4-C^{14} per sq. cm.).

In the experiments with lens paper, the reference value was determined by extrapolating the self-absorption curve to zero weight. It was observed that the activity values obtained with "weightless" samples (less than 0.1 γ per sq. cm.) of cortisol-4-C^{14} on lens paper fell on this curve when plotted at the thickness of the lens paper—i.e., 1 mg. per sq. cm. (Figure 2). Use of liver tissue instead of muscle in the formamide procedure gave essentially identical self-absorption curves.

Statistical Calculations. The counting error was calculated according to established procedures (*2*). The samples were counted to give standard deviations of counting rates as listed in Table I. The same table gives the over-all standard deviation which was calculated from the experimental data. This expression (Table I line 1) for the significance of the data includes the errors caused by handling, pipetting, plating, etc., in addition to the inherent statistical deviations of the counting rate (Table I, lines 2 and 3).

The curves shown in the figures were determined by the method of least squares. The self-absorption values were expressed by No/N as the ordinate with the sample thickness (milligrams per square centimeter) as the abscissa, where No = activity in a "weightless" sample, and N = activity observed at various thicknesses. This method of plotting the "self-absorption factor," No/N, as employed in the figures, is considered useful, because the absorption-free activity can be obtained directly from the observed activity by multiplication with this factor.

RESULTS AND DISCUSSION

On the basis of their original observation, Lahr, Olsen, Gleason, and Tabern (*6*) have developed a method for radioactivity measurements in tissue by homogenizing whole organs and dissolving aliquots in formamide. The beta activity of gold-198, phosphorus-32, and yttrium-90 was thus determined by liquid counting at infinite thickness. This procedure could not be utilized in the studies of the present authors, where carbon-14 must be determined in many hundreds of organs and tissues of small laboratory animals (*3*), making homogenization of the individual samples impractical.

The results of the self-absorption measurements for the cortisol-4-C^{14}–muscle-formamide preparations are given in Figures 1 and 2. The abscissas of these figures show that all determina-

Table I. Standard Deviation of Carbon-14 Determinations with and without Lens Paper

(Compiled from experimental data of Figures 1 and 2)

	400 C.P.M. Level, %		40 C.P.M. Level, %	
	With lens paper	Without lens paper	With lens paper	Without lens paper
1. Over-all standard deviation ±	7.1	11.2	5.9	14.6
2. Standard deviation of counting rate ±	1.6	1.6	...	...
3. Standard deviation of counting rate ±	...	...	3.1–7.5	3.2–10.2

1. $\sqrt{\frac{\Sigma x^2}{n-1}}$ (*11*).
2. $\sqrt{C_s}$ Formula for large counts compared to background (*2*).
3. $\sqrt{\frac{C_s}{T_s^2}+\frac{C_b}{T_b^2}}$ Formula for counts not greatly different from background (*2*).

Table II. Self-Absorption Factors for Carbon-14–Labeled Substances

(Factors are given as reciprocals and are accurate within the range of error of the corresponding self-absorption values.)

Substance	Thickness, *t*, Mg./Sq. Cm. 5	10	15	20
"Theoretical" (*7*)	1.72	2.78	3.70	4.35
Barium carbonate (*16*)	1.90	3.04	4.35	5.68
Barium carbonate (wet ground) (*15*)	1.51	2.18	2.95	3.85
Barium carbonate (dry ground) (*15*)	1.49	1.92	2.56	3.17
Glucose (amorphous) (*15*)	1.54	1.92	2.33	2.78
Glucose (crystalline) (*15*)	1.61	2.27	3.17	4.17
Fatty acids (*16*)	1.89	2.95	4.00	5.25
Wax (*17*)	2.39	3.85	5.55	6.25
Muscle preparations with lens paper	2.15	3.30	4.45	5.60
Muscle preparations without lens paper	3.74	5.44	7.04	8.84

tions were done at sample thicknesses lower than infinite thickness, which has been found for organic substances to range from 20 mg. per sq. cm. (fatty acids) to 25 to 30 mg. per sq. cm. (glucose) (*15*).

Figure 1 shows that the self-absorption observed with the muscle-formamide procedure appears to approximate a linear proportionality to the sample thickness. It is known that the self-absorption of β-radiation in sample material does not necessarily follow an exponential curve (*1, 5*). The equation that fitted the experimental observations best was found to be $No/N = 2.04 + 0.34t$, where t equals sample thickness in milligrams per square centimeter. This equation and the curve of Figure 1 indicate that the apparent "specific activity" of the samples rises sharply as the sample thickness approaches zero, an effect which presumably is caused by backscattering from the aluminum planchet. In this range of very low thicknesses (broken line in Figure 1) application of the curve of Figure 1 is not recommended; use of lens paper is considered preferable.

The self-absorption curve obtained with the use of lens paper (Figure 2) leads through $No/N = 1$ at zero thickness. This is the consequence of the fact that the value of zero thickness was not determined by measuring weightless samples but rather by extrapolating the curve to zero thickness. The weights given on the abscissa in Figure 2 include the weight of the lens paper, which was approximately 7 mg. per planchet. The use of lens paper has the advantage of reducing the spreading of the observations, as indicated by lower standard deviations (Table I).

The self-absorption curve in the presence of lens paper followed the equation $No/N = 1 + 0.23t$. A comparison with the exponential curve $No/N = \mu t/1 - e^{-\mu t}$ (*2*), where $\mu = 0.31$ (Figure 2), indicates better agreement of the observed values with the linear curve.

Table II shows self-absorption factors for a number of carbon-14–labeled compounds. They are given as the reciprocal of N/No, the percentage of the "zero weight"—i.e., self-absorption-free activity—observed at certain thicknesses. The "theoretical" values were calculated from the exponential formula given by Libby (*7*). In the last two lines of Table II are listed the self-absorption factors shown in Figures 1 and 2 of the present report. The correction factors observed for the muscle preparations without lens paper are comparatively large. This follows from the fact that the "true zero" values in this case (Figure 1) were determined at extremely low thicknesses.

It is evident from Table II that the self-absorption factors do not show any consistent relationship with the chemical nature of the compounds. The differences observed are presumably caused by a different physical arrangement of the samples and other variations in the counting conditions. These observations emphasize the necessity of determining experimentally the self-absorption correction factors for the specific conditions of any experimental setup (*9*).

Application of the procedure in studies on the distribution of cortisol-4-C^{14} in rats (*3*) showed that the recovery of carbon-14 was essentially quantitative. Ten animals were injected 50,000 to 58,000 c.p.m. (approximately 12.5 to 14.5 γ) of this steroid, and 5 minutes later a complete analysis was made of practically all organs and tissues. The average recovery in the 10 rats was found to be 105.6%, S.D. = ±10.1%. The over-all reproducibility of single observations follows from the data of Table I. The weight of the dried organs or tissues that were dissolved in formamide ranged between 3 mg. and about 40 grams. The sample weights on the aluminum planchets were as low as 3 mg., but preferably between about 40 and 60 mg. (approximately 5 to 9 mg. per sq. cm.).

The outlined procedure permits a comparatively rapid determination of radiocarbon in animal tissues. It was developed for cortisol-4-C^{14} which did not show any loss of radioactivity when heated in formamide at 210° C. or when dried under an infrared lamp. The method appears to be applicable to other carbon-14 compounds, provided a check is made to ascertain that no loss of radioactivity occurs during the various experimental operations.

ACKNOWLEDGMENT

The authors are indebted to the Endocrinology Study Section, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, for supplying the cortisol-4-C^{14} through Tracerlab, Inc., Boston 10, Mass.

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RECEIVED for review April 18, 1956. Accepted July 16, 1956.

Determination of Di- and Trialkyl Phosphites in the Presence of Each Other

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In an alcoholic caustic solution dialkyl phosphites are rapidly hydrolyzed to form sodium monoalkyl phosphites, with no interference from trialkyl phosphites. In an acidic alcoholic solution trialkyl phosphites are readily hydrolyzed to form dialkyl phosphites, which may then be hydrolyzed with caustic. Both reactions are stoichiometric and consume one mole of caustic per mole of phosphite.

TRIALKYL phosphites are generally prepared by the reaction of alcohol and phosphorus trichloride in the presence of a base (Equation 1); without a base, dialkyl phosphites are formed (Equation 2). In the commercial preparation of trialkyl phosphites some dialkyl phosphites are usually formed. Therefore, to determine the purity of trialkyl phosphites, it is necessary to determine each of the two components in the mixture.

Relatively few such chemical procedures are reported in the literature. One procedure, which is specific for the dialkyl phosphite content, is the nonaqueous titration of weak acids (*2*). Another procedure for purity only is the determination of molecular weight with alcoholic potassium hydroxide (*3*). Iodine solutions have also been used to indicate purity (*5*). There are very few published data on the two latter procedures.

The method presented here takes advantage of the rapid hydrolysis of alkyl phosphites. In an alkaline alcoholic medium dialkyl phosphites are instantly hydrolyzed to form sodium monoalkyl phosphites, (Equation 3), while trialkyl phosphites react very slowly (*1*, *4*). The rates of reaction of the two components are far enough apart to permit determination of dialkyl phosphites in the presence of trialkyl phosphites by adding an excess of alcoholic sodium hydroxide and immediately titrating the excess caustic with standard acid. In an alcoholic acid medium trialkyl phosphites are very rapidly hydrolyzed to form dialkyl phosphites (*1*) (Equation 4). The phosphite is then all present as dialkyl phosphite and may be determined by alkaline hydrolysis.

$$3ROH + PCl_3 + 3\ base \rightarrow (RO)_3P + 3\ base.HCl \quad (1)$$

$$3ROH + PCl_3 \rightarrow (RO)_2\overset{O}{\overset{\|}{P}}{-}H + RCl + 2HCl \quad (2)$$

$$(RO)_2\overset{O}{\overset{\|}{P}}{-}H + NaOH \rightarrow (RO)(NaO)\overset{O}{\overset{\|}{P}}{-}H + ROH \quad (3)$$

$$(RO)_3P + HOH \xrightarrow{acid} (RO)_2\overset{O}{\overset{\|}{P}}{-}H + ROH \quad (4)$$

EXPERIMENTAL

It was found that in an alcoholic medium 1 mole of dialkyl phosphite consumes 1 mole of sodium hydroxide after 1 minute, and this value remains constant for at least 1 hour. Trialkyl phosphite, ranging from ethyl to octyl, consumes no sodium hydroxide for as long as 10 minutes in an alcoholic medium. After 10 minutes of hydrolysis in a slightly acidic alcoholic medium, 1 mole of trialkyl phosphite consumes 1 mole of sodium hydroxide after 1 minute, and this value remains constant for 1 hour. All these reactions were carried out at room temperature.

INTERFERENCES

Alcohols, amine hydrochlorides, and dialkyl alkanephosphonates (the rearrangement isomer of trialkyl phosphites) do not interfere with this method.

If acidic compounds such as monoalkyl phosphites, or basic compounds such as amines, are present, they can be compensated by neutralizing the alcoholic solution of the sample with 0.1*N* acid or base.

PROCEDURE

Dialkyl Phosphite. Dissolve 1 to 2 grams of trialkyl phosphite in 50 to 100 ml. of dry ethyl alcohol. Neutralize with 0.1*N* sodium hydroxide to a light pink with phenolphthalein indicator. Add 20.0 ml. of 0.1*N* sodium hydroxide, mix, add about 10 ml. of a 4% solution of boric acid, and back-titrate with 0.1*N* hydrochloric acid until the solution is colorless. Each mole of sodium hydroxide consumed is equivalent to 1 mole of dialkyl phosphite. Sample size and excess caustic may be regulated to suit the particular sample that is to be analyzed. Dialkyl phosphites may also be assayed by this procedure. The boric acid is added to buffer the solution, thus preventing local hydrolysis of the trialkyl phosphite when back-titrating with acid.

Table I. Typical Results with Synthetic Mixtures

% Di		% Tri[a]	
Added	Found	Added	Found
Di- and Tributyl Phosphites			
1.0	0.9	99.0	99.1
3.0	3.0	97.0	97.0
5.0	5.1	95.0	94.7
10.0	9.9	90.0	89.9
15.0	14.8	85.0	84.8
20.0	19.7	80.0	80.2
Di- and Triiso-octyl Phosphites			
7.0	7.0	93.0	92.7
10.0	9.8	90.0	90.2
15.0	14.7	85.0	84.8
20.0	19.9	80.0	79.8

[a] Standard deviation of tri-component, 2 parts per thousand.

Total Phosphite. Dissolve a sample containing 0.002 to 0.003 mole of phosphite in 100 ml. of 95% ethyl alcohol. Neutralize immediately to a light pink with phenolphthalein indicator. Add 5.0 ml. of 0.1*N* hydrochloric acid, mix, and allow to sit for at least 10 minutes. Add 40.0 ml. of 0.1*N* sodium hydroxide, mix, and titrate with 0.1*N* hydrochloric acid until the solution is colorless. The net moles of caustic consumed are equivalent to the moles of phosphite present. The trialkyl phosphite content is calculated by subtracting the dialkyl phosphite from the total phosphite. Sample size, strength of solutions, and the amount of alcohol used as solvent may be regulated as desired. The authors prefer using aqueous acid and base with enough alcohol to keep a clear solution rather than alcoholic acid or base.

RESULTS

The method is simple, rapid, and accurate. It is applicable to alkyl phosphites ranging from ethyl to octyl alkyl groups, with little interference from materials commonly found in commercial trialkyl phosphites. Table I shows typical results obtained from synthetic mixtures of pure fractionated di- and tributyl and di- and triiso-octyl phosphites.

ACKNOWLEDGMENT

The authors wish to express their thanks to Betty Bruns for assisting in compiling the analytical data.

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RECEIVED for review May 19, 1956. Accepted July 10, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956.

Decomposition of Organic Fluorine Compounds by Wickbold Oxyhydrogen Flame Combustion Method

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The Wickbold oxyhydrogen combustion method is an excellent way of decomposing organic fluorine compounds for determination of fluorine. In this method the vaporized organic fluoride or its partial combustion products are passed through an oxyhydrogen flame which decomposes the sample to carbon dioxide and hydrogen fluoride. The hydrogen fluoride is absorbed in dilute sodium hydroxide solution and subsequently titrated with thorium nitrate. This method is rapid and eliminates the difficulty resulting from high salt content normally present after a Parr bomb-type decomposition.

OVER 500 references have been listed by McKenna (*2–4*) on methods of decomposition and analysis of fluorine compounds. However, none of the procedures given in the above reference are entirely satisfactory.

The two main sources of error encountered in fluorine analysis are:

(1) Difficulty in obtaining quantitative conversion of the organic fluorine to ionic fluoride. It is usually necessary to employ more drastic decomposition conditions than those required for other organic compounds, and even then some organic fluorides give low results due to incomplete decomposition.

(2) The quantitative determination of fluoride in the decomposed sample. Volumetric methods based on formation of complexes and precipitates are complicated by the difficulties of end point detection, slow reactions, and general nonstoichiometric conditions caused by pH and salt effects. Gravimetric determinations generally are not suitable because of the solubility of metallic fluorides and the difficulty in filtering precipitates.

Probably the most widely used method for fluorine analysis is a Parr bomb decomposition, followed by titration of the fluoride with thorium nitrate using alizarin red as indicator. A Parr bomb method has proved satisfactory in this laboratory for the decomposition of most organic fluorides; only in the case of highly volatile compounds and compounds with high fluorine content has there been difficulty in the decomposition step. The nature of this decomposition, however, makes the final determination of fluoride difficult because of the large amount of salt residue from the Parr bomb fusion. This high salt content not only has a detrimental effect on the sharpness of the end point, but also changes the stoichiometry of the reaction.

In an effort to avoid these difficulties, a study has been made of the decomposition of organic fluorides by a Wickbold-type, oxyhydrogen flame combustion apparatus (*5, 6*).

APPARATUS

The quartz flame combustion apparatus (Figure 1) is similar to that described by Wickbold (*5*) and consists of five main parts: the vaporization tube, *A*, the burner, *B*, the flame chamber, *C*, the condenser, *I*, and the absorption receiver, *D*. Absorption receiver and Reitmeyer attachment, *E*, are of borosilicate glass; all other parts are of quartz or Vycor (96% silica glass) glass. The vaporization tube is the section in which the sample is heated to give partial combustion or vaporization. Gaseous products then pass from the vaporization tube via a capillary tube into the burner head at *B*. The oxygen and hydrogen are supplied to the burner from the two tubes, *x* and *y*, so that the capillary from the vaporization tube is surrounded by the hydrogen and oxygen tubes which end at the burner head.

The burner section is connected to the flame chamber, *C*, by a 19/38 joint at *B*. The flame chamber is surrounded by a water jacket to prolong the life of the quartz tube and to prevent possible melting of the quartz. The flame chamber tapers down at the end to about 6 mm. and is connected to a spiral enclosed in a water condenser, which in turn is connected at *J* to the absorption receiver with a 10/30 joint. The apparatus is mounted on a Transite panel. The flows of the flame oxygen, sweep oxygen, hydrogen, and exhaust lines are controlled by needle valves, mounted on the front of the panel, which are connected to the Brooks multitube Flowmizer flow meters mounted in a single four-unit mount. The apparatus is surrounded by a Lucite acrylic resin safety shield.

A probe burner, $1^1/_2 \times 20$ cm., *P*, is made of Vycor glass. The oxygen is supplied to the burner through the outer tube and the gas by the inner tube, both of which taper down at the burner head.

A platinum boat, $1 \times 4 \times 1$ cm., was used in the vaporization of solid samples and high boiling liquids. A quartz pig similar to that used by Wickbold was used for the volatile liquid samples.

The illuminator for the titrations is an H-3, 85-watt Westinghouse mercury lamp with a vapor lamp transformer. The lamp is housed in a mount that directs the ultraviolet light at an angle

to the titrating vessel and screens out strong light from other portions of the working area.

REAGENTS

Thorium Nitrate Solution, 0.1*N*. Dissolve 14.0 grams of C.P. thorium nitrate tetrahydrate in water and dilute to 1 liter. Standardize this solution with C.P. sodium fluoride which has been dried for 2 hours at 110° C.

Monochloroacetic Acid Buffer, 0.50*M*. Dissolve 23.6 grams of reagent grade monochloroacetic acid in 400 ml. of water, add 1.0*N* sodium hydroxide to this solution until the pH is 3.0, cool, and dilute to 500 ml. Store in a polyethylene bottle. This buffer solution should not be stored more than 1 or 2 weeks.

Standard Sodium Fluoride Solution. Weigh out 2.2105 grams of C.P. sodium fluoride (dried for 2 hours at 110° C.), dissolve in water, and dilute to 1 liter. Store in a polyethylene bottle. This solution contains 1.00 mg. of fluoride per ml.

Sodium Alizarin Sulfonate Indicator. Prepare a 0.05% solution and make alkaline by adding 0.1*N* sodium hydroxide dropwise until the color changes to a deep red.

PROCEDURE

The absorption receiver is filled with 50 ml. of 0.1*N* sodium hydroxide and the flame ignited after first removing the burner, *B*, from the standard taper joint. It is then possible, by applying a vacuum at *E*, to return the lighted burner to the flame chamber, *C*, so that the chamber is nearly filled by the oxyhydrogen flame. The various flow rates are important at this point and are adjusted to predetermined values with the aid of the flow meters. Although the exact flow rates will vary to a certain extent with the apparatus, the following rates were found most suitable for the author's apparatus: a flow setting of 6.0 to 7.2 liters per minute for the flame hydrogen, *y*, 5.0 to 6.0 liters per minute for the flame oxygen, *x*, 1.6 to 2.2 liters per minute for the sweep oxygen, *G*, and 4.0 to 4.5 liters per minute for the exhaust line, *E*.

The sample is introduced into the vaporization tube through the removable joint at *F*. Oxygen is passed over the sample from the sweep oxygen line, and the sample is heated with a Fisher burner until vaporization or partial combustion takes place. The oxygen sweeps the vaporized sample through the capillary tube and into the flame chamber. The temperature in this chamber is around 2000° C. and all carbon-fluorine bonds are broken with the formation of carbon dioxide and hydrogen fluoride. The hydrogen fluoride is swept through the condenser coil into the receiver, where it is absorbed by the aqueous scrubbing solution. The continuous formation of water vapor from the reaction of the hydrogen and oxygen assures complete washing of the flame chamber and condenser of adsorbed hydrogen fluoride. The fluoride solution is then removed from the absorption receiver and diluted to 200 ml.

An aliquot of this fluoride solution containing 5 to 9 mg. of fluoride is then added to a 210-ml. casserole along with 1.00 ml. of the alizarin red indicator, 2.00 ml. of 0.50*M* monochloroacetic acid buffer, and enough water to make approximately 100 ml. The pH of the solution is adjusted to 3.4 with 0.3*N* hydrochloric acid or 0.3*N* sodium hydroxide and the final volume made approximately 103 ml. This solution is then titrated with standard thorium nitrate until a slight color change from yellow to pink appears under a white light. At this point the titration is continued in the dark with the ultraviolet light source directed down toward the casserole at a 45° angle. A definite appearance of a faint pink color is now taken as the final end point.

Liquid samples can be treated similarly. The sample is weighed in a quartz pig with a ground-glass stopper, which is removed as the sample is placed in the vaporization tube. The sample is gently heated until it has been completely vaporized into the flame chamber.

Gaseous samples can be decomposed by connecting the gas sample tube directly to the vaporization tube and slowly sweeping the gas through the vaporization tube into the burner. The combustion of gaseous samples requires a much slower rate of flow for the sweep oxygen. The most convenient procedure was to allow the sample to diffuse into the burner by the slight negative pressure of the vaporization tube and combustion chamber. After 1 to 2 minutes a slow stream (0.1 to 0.2 liter per minute) of sweep oxygen is allowed to flow through the gas sample tube for 2 to 3 minutes, after which the sweep oxygen is increased to the normal value of 1.6 to 2.2 liters per minute until the gas is completely displaced from the sample tube.

In the decomposition of some solids and liquids, considerable amounts of tar may form during the vaporization process and collect in the capillary between the vaporization tube and the burner end. Large amounts of this tar can give low results in the final fluoride analysis, as well as be troublesome to clean out. An all-Vycor oxyhydrogen flame probe, *P*, provided a rapid and effective means for solving this problem. The usual procedure was to remove the boat from the vaporization tube after the vaporization and, with the oxyhydrogen flame still in the flame chamber, increase the vacuum slightly and direct the probe flame into the capillary tube through the joint at the end of the vaporization tube. The vacuum caused the probe flame to be drawn through the capillary tube, thus removing all the tar and carbon in less than 15 seconds. Care must be exerted at this point to prevent melting of the capillary tube.

DISCUSSION

The results of analyses of several fluorine compounds are given in Table I.

In most cases the results tend to be slightly on the low side; this is attributed to a possible boron effect from the borosilicate glass absorption receiver. Addition of mannitol to the sample was found to give a slight increase in acidity, thus indicating the presence of boron. Because this error was small and reproducible, a recovery factor of 1.006 was adopted for use on all known samples.

It is interesting that quantitative decompositions took place even with trifluoromethane and tetrafluoromethane. These compounds have been notorious for their stubbornness in resisting decomposition. Freier and coworkers (*1*) recently reported that, in the quartz tube combustion method, only about 3% of the theoretical fluorine content could be obtained in the decomposition of tetrafluoromethane even in the presence of water vapor. The quantitative decomposition of tetrafluoromethane is more important than it appears on the surface, for in many combustion methods it is thought that the cause of low results is the formation of tetrafluoromethane. With the Wickbold method, com-

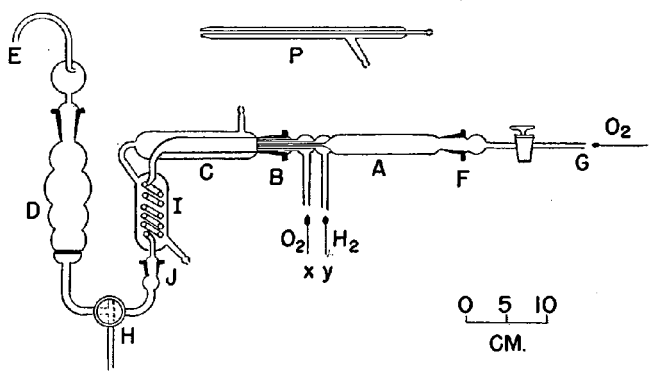
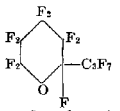


Figure 1. Oxyhydrogen combustion apparatus

A. Vaporization tube
B. Burner
C. Flame chamber
D. Absorption receiver
E. Reitmeyer attachment
F. Removable joint
G. Sweep oxygen inlet
H. 3-way stopcock
I. Spiral condenser
J. 10/30 joint
P. Probe burner
x. Burner oxygen inlet
y. Burner hydrogen inlet

Table I. Results of Analyses with Wickbold Apparatus

Substance	% Fluorine Theory	Found
Teflon (tetrafluoroethylene resin)	76.0	75.84 75.69 75.35 75.76
Sodium trifluoroacetate	41.9	41.8
Fluorobenzene	19.77	19.67 19.60 19.50
Trifluoroacetylacetone	37.0	36.5
1-Chloro-3-fluorobenzene	14.55	14.14 14.58
Perfluoro-(2-*n*-propyl)-pentamethylene oxide	73.10	73.46 73.29
		
n-Heptafluorobutyric acid	62.18	60.83 61.88
Dichlorodifluoromethane	31.4	31.1 31.4
Trifluoromethane	81.4	81.3
Tetrafluoromethane	86.4	86.0 85.8

plete combustion of tetrafluoromethane takes place, and, consequently, similar results on other samples which have been difficult to decompose should be expected.

The data in Table I indicate that even the sodium salt of trifluoroacetic acid is quantitatively decomposed. In this case the salt was first decomposed by the usual method, during which about two thirds of the actual fluorine was liberated. The boat was then removed from the apparatus and washed with water to remove the residual sodium fluoride, which was then combined with the solution from the absorption receiver and made up to volume. This procedure should work for all salts that form nonvolatile soluble fluorides, and with possible modification should be of use with other fluoro-organic salts.

Some work has been done on the effect of sulfur on the determination of fluorine. Although no standard compounds which contained both sulfur and fluorine were available, results by the Wickbold method on unknown samples which contained substantial amounts of sulfur were 1 to 3% lower than the corresponding Parr bomb results. These low results are probably caused by a change in the stoichiometry of the thorium nitrate–fluoride reaction and are not due to the decomposition procedure.

The Wickbold flame combustion method has several advantages over the more conventional methods. First, the method is rapid and gives excellent results with solid, liquid, or gaseous samples. The case of analysis of gases makes the method ideal for elemental analysis of such samples. The actual combustion time for 0.1- to 0.2-gram samples is only 3 to 7 minutes, and the total time for an analysis is 15 to 20 minutes. One of the most important features of the method is that it eliminates the large amount of salt left by most decomposition methods.

ACKNOWLEDGMENT

The author wishes to acknowledge the development by E. S. Wilkins of this laboratory of the original procedure for determination of the alizarin red end point by the use of ultraviolet light from an H-3 mercury lamp.

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RECEIVED for review February 20, 1956. Accepted June 22, 1956. Contribution No. 383, Chemical Department, Experimental Station, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. Presented at 8th Annual Delaware Chemical Symposium, University of Delaware, Newark Del., February 18, 1956.

Analytical Applications of the Hanging Mercury Drop Electrode

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The hanging drop electrode (a stationary spherical mercury electrode) has been applied to the reduction of zinc, cadmium, lead, and thallous ions. Voltammetry with continuously varying potential using this new electrode is very rapid and reproducible, and is more sensitive than conventional polarography. The electrode appears to be a useful new analytical tool.

THE use of stationary electrodes in volammetry offers several distinct advantages over the dropping mercury electrode. In addition to the reduced charging current, the constant area of the electrode makes it possible to scan the current-voltage curve in a relatively short time.

Stationary platinum electrodes have been studied by Laitinen and Kolthoff (*5*), Rogers, Miller, Goodrich, and Stehney (*8*), and Nicholson (*7*), among others. Mercury pool electrodes were investigated by Streuli and Cooke (*10*), who reported increased sensitivity (due to the large electrode area) and lower charging currents. Unfortunately, it is difficult to reproduce the area of mercury pool electrodes, because the solution has a tendency to creep along the walls of the mercury container. Furthermore, rather large amounts of mercury are consumed, as the electrode must be renewed frequently.

The development of the hanging drop electrode by Gerischer (*3*) and Berzins and Delahay (*1*) has made available a very reproducible stationary spherical mercury electrode which shows great promise as an analytical tool. The solution of the equations for spherical diffusion with continuously varying potential by Frankenthal and Shain (*2*) provides a reliable and accurate interpretation of the experimental results.

As examples of the analytical applications of this electrode, the reduction of four ions and a mixture of two of them was studied.

EXPERIMENTAL

Apparatus. A mechanical sweep generator, direct current bias circuit, and load resistor similar to the circuits described by Shain and Crittenden (*9*) were placed in series with the cell. The internal resistance drop across the load resistor was amplified by a Millivac DCA-3 amplifier. The output of this amplifier was recorded as a function of time on a Leeds & Northrup Type G Speedomax recorder (10-mv. full scale, 2-second response).

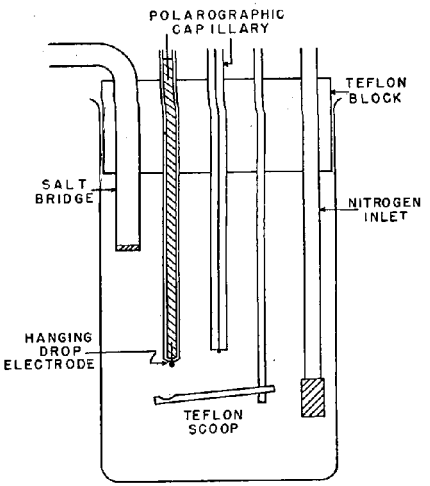

Figure 1. Cell and electrode assembly

The cell and electrode assembly are shown in Figure 1. The cell consisted of a 300-ml. beaker connected to a saturated calomel electrode by means of a salt bridge containing the indifferent electrolyte. The cell resistance was 300 ohms. The cell was not thermostated, except for those experiments where the results were to be compared to theory, when the cell and reference electrode were thermostated at 25.0° ± 0.1° C. Nitrogen was bubbled through the solution by means of a gas dispersion tube, which could be raised so that the nitrogen would flow over the solution during the recording period. The mercury drops were produced by a conventional polarographic capillary assembly, and were transferred to the working electrode by means of a movable Teflon scoop. A capillary with a drop time of 5 seconds was used. Capillaries with longer period sometimes produced drops of varying size when vibration dislodged the drops prematurely.

The hanging drop electrode was constructed by sealing a short platinum wire (0.254 mm. in diameter) in the end of a piece of soft-glass tubing. The platinum was cut off as short as possible, filed flush with the glass, and plated with mercury, so that the drops would adhere.

The construction and plating of the working electrode were fairly critical, especially in studying very dilute solutions. The platinum had to be completely covered to prevent the appearance of small erratic hydrogen reduction waves. When the electrode was drawn to a rather sharp point (which increased the accessibility of the top of the drop), microscopic cracks often appeared in the glass, allowing small amounts of solution to come in contact with the platinum wire. When the platinum was allowed to project slightly from the glass, it was difficult to plate completely; this again led to erratic residual currents.

The plating was done in a concentrated mercuric nitrate solution, 1*N* in nitric acid, using a mercury pool anode. With a potential of 1.5 volts across this plating cell, 5-minute plating time was sufficient. It was usually necessary to reduce the platinum oxide film with an acid solution of ferrous sulfate before plating the electrode, so that the surface would be uniformly covered. The electrode had to be replated occasionally, especially after standing in air for several days.

By using a working electrode of the above dimensions, 1, 2, or 3 drops of mercury could be collected. In all work reported here 2 drops of mercury were used. After each current-voltage curve was recorded, the mercury was dislodged and 2 new drops were collected to form a fresh hanging drop electrode. The size of the hanging drop electrode was calculated from the weight of a known number of drops. The radius was 0.0678 cm. and the area was 0.0577 sq. cm.

Materials. All chemicals were reagent grade and normally were used without further purification. For work with very dilute solutions (below $10^{-5}M$), the indifferent electrolyte was purified by electrolysis with a mercury pool cathode (*6*). All solutions were prepared with doubly distilled water.

Traces of metallic impurities in the mercury caused high residual currents. For this reason triply distilled mercury was redistilled from an all-glass system.

Linde high purity nitrogen was used without further purification to remove oxygen from the solutions.

RESULTS AND DISCUSSION

The analytical procedures used with the hanging drop electrode are very similar to those encountered in conventional polarography. The main difference is that the voltage span is traversed in 20 to 50 seconds, rather than the 10 to 15 minutes used in most automatic polarographs. This rapid scanning of the potential with a stationary electrode results in the characteristic peaked current-voltage curve shown in Figure 2. The peak is caused by the depletion of the reducible species in the diffusion layer surrounding the electrode. Shorter scanning times can be used, if the response time of the associated equipment (recorder in particular) is fast enough. Longer scanning times usually lead to erratic results, due to convection around the electrode surface as the diffusion layer extends further into the solution. Two rates of voltage change were used in this work: 0.0139 and 0.0278 volt per second, corresponding to 36 seconds for 0.5- and 1.0-volt sweeps.

As examples of the analytical applications of this method of analysis, current-voltage curves were obtained for the reduction

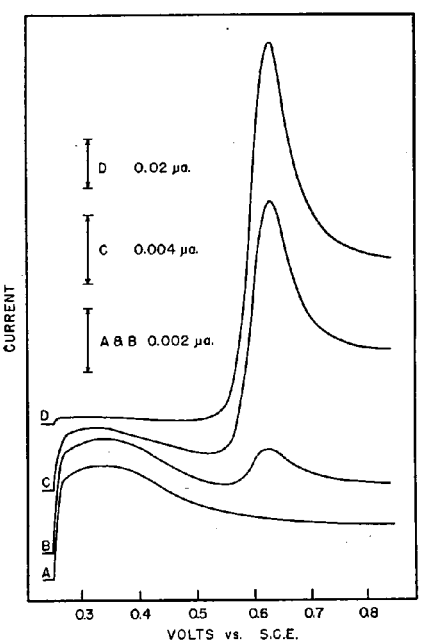

Figure 2. Current-voltage curves for reduction of cadium in 0.1*M* potassium chloride

***A*. Residual current**
***B*. 1.00 × $10^{-6}M$ cadmium**
***C*. 1.00 × $10^{-5}M$ cadmium**
***D*. 1.00 × $10^{-4}M$ cadmium**

of cadmium, zinc, lead, and thallous ions, and for a mixture of zinc and cadmium.

Residual Currents. If the mercury and the indifferent electrolyte are purified, residual currents encountered with the hanging drop electrode are primarily charging current due to the high rate of voltage change. These charging currents were very reproducible and amounted to about 3×10^{-8} ampere in the region of the electrocapillary maximum (Figure 2). This current was negligible in solutions more concentrated than $10^{-5}M$, but a correction had to be applied to the more dilute solutions. This was most easily accomplished by obtaining current-voltage curves for a blank solution containing only the indifferent electrolyte, and subtracting the measured residual current from the analytical curves.

Single-Component Systems. A set of typical current-voltage curves for the reduction of cadmium ion is shown in Figure 2. Similar sets of current-voltage curves were obtained for each of the other ions studied. In order to check the reproducibility of the curves, six determinations were made on each solution, using a new hanging drop electrode for each. The results are summarized in Table I.

The reproducibility of replicate determinations on the same solution equals or exceeds conventional polarography for $10^{-5}M$ or more concentrated solutions. At concentrations below $10^{-5}M$ the peak current may become less than half of the total current, and random errors in subtracting the charging current account for the larger deviation. At concentrations below $10^{-6}M$ the indeterminate errors increase rapidly. Nevertheless, it is possible to detect the presence of a reducible ion at concentrations as low as $5 \times 10^{-8}M$.

Table I. Peak Current as a Function of Concentration

Solution						
Ion	Concn., mole/liter	Peak Current[a], Ampere	Average[a] Deviation, %	Peak Current[b] Concn., μa / Mmole/Liter	Average Deviation, %
Cd^{++}	1.00×10^{-6}	1.47×10^{-8}	2.5		
(0.1M	1.00×10^{-5}	1.49×10^{-7}	0.9	14.8	
KCl)	1.00×10^{-4}	1.50×10^{-6}	0.5		1.0
	1.00×10^{-3}	1.46×10^{-5}	0.5		
Zn^{++}	1.00×10^{-6}	1.44×10^{-8}	6.3		
(0.1M	1.00×10^{-5}	1.41×10^{-7}	0.5		
KNO_3)	1.00×10^{-4}	1.45×10^{-6}	0.5	14.2	2.1
	1.00×10^{-3}	1.36×10^{-5}	0.5		
Tl^{+}	1.00×10^{-6}	0.942×10^{-8}	2.5		
(0.1M	1.00×10^{-5}	0.918×10^{-7}	0.6		
KNO_3)	1.00×10^{-4}	0.932×10^{-6}	0.5	9.29	0.9
	1.00×10^{-3}	0.923×10^{-5}	0.5		
Pb^{++}	1.00×10^{-6}	1.69×10^{-8}	1.8		
(0.1M	1.00×10^{-5}	1.67×10^{-7}	1.2		
KNO_3)	1.00×10^{-4}	1.72×10^{-6}	0.5	16.8	1.5
	1.00×10^{-3}	1.64×10^{-5}	0.5		

[a] Average and average deviation of six determinations on the same solution.
[b] Average of peak current/concentration for range 10^{-6} to $10^{-3}M$.

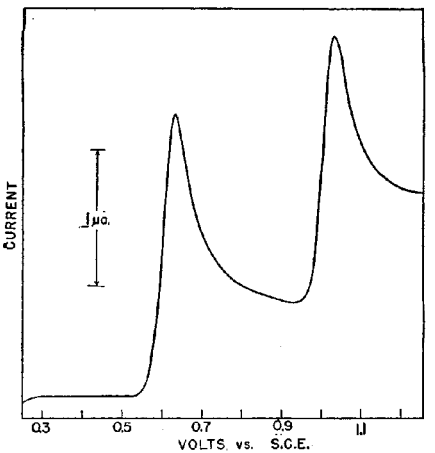

Figure 3. Current-voltage curve for reduction of $1.00 \times 10^{-4}M$ cadmium and $1.00 \times 10^{-4}M$ zinc in 0.1M potassium chloride

The last two columns in Table I indicate that the peak current is a linear function of concentration over a rather wide range of concentrations. Above $10^{-3}M$ the sensitivity decreases, owing to the internal resistance drop in the cell, which results in a nonlinearity in the rate of voltage change.

Two-Component Systems. A typical current-voltage curve obtained on a solution containing $10^{-4}M$ cadmium and $10^{-4}M$ zinc is shown in Figure 3. In a case such as this the concentration of the species most easily reduced (cadmium in this case) may be determined as accurately as in a single-component system. On the other hand, the peak current for the second substance (zinc) is strongly influenced by the diffusion current of the first. In order to determine this effect, nine different solutions were analyzed, in which the cadmium and zinc concentrations were 10^{-3}, 10^{-4}, and $10^{-5}M$ in all combinations. The rate of voltage change was 0.0278 volt per second. A summary of the results (Table II) shows that the presence of the zinc has no effect on the cadmium determinations.

The determination of zinc is more difficult and depends on the ratio of zinc to cadmium. Correction for the diffusion current of the cadmium may be accomplished two ways. When the zinc concentration is equal to or greater than the cadmium concentration, the entire analysis can be obtained from a single current-voltage curve. The effect of the cadmium diffusion current is subtracted by using a pure cadmium solution (of the same

Table II. Two-Component Systems

Concn., Mole/Liter[a]		Peak Current[b]					
Zn^{++}	Cd^{++}	Zn^{++}[c]	Av. dev., %	Zn^{++}[d]	Av. dev., %	Cd^{++}[c]	Av. dev., %
1.00×10^{-3}	1.00×10^{-3}	1.86×10^{-5}	0.5	1.83×10^{-5}	1.5	1.91×10^{-5}	0.5
	1.00×10^{-4}	1.84×10^{-5}	0.5			2.01×10^{-6}	0.5
	1.00×10^{-5}	1.84×10^{-6}	0.5			2.01×10^{-7}	0.6
1.00×10^{-4}	1.00×10^{-3}			1.96×10^{-6}	3.6	1.91×10^{-5}	0.5
	1.00×10^{-4}	1.99×10^{-6}	1.5	1.92×10^{-6}	1.9	2.04×10^{-6}	0.5
	1.00×10^{-5}	1.93×10^{-6}	1.2			2.03×10^{-7}	0.8
1.00×10^{-5}	1.00×10^{-3}					1.91×10^{-5}	0.5
	1.00×10^{-4}			2.00×10^{-7}	3.1	2.04×10^{-6}	0.5
	1.00×10^{-5}	1.98×10^{-7}	1.2	1.93×10^{-7}	1.1	2.04×10^{-7}	0.7

[a] Indifferent electrolyte 0.1M KCl.
[b] Average and average deviation of six determinations on same solution.
[c] Current-voltage curve obtained by single scan of potential range.
[d] Current-voltage curve for Zn^{++} obtained after decay of Cd^{++} current.

concentration as in the mixture) as a blank. The results using this type of compensation are given in the first column of zinc currents in Table II. It is not possible to use this method, however, when the concentration of cadmium is greater than of zinc.

When the second peak was much smaller than the first, the best way to compensate for the cadmium diffusion current was to set the starting potential slightly more cathodic than the first peak. When the current due to the cadmium reduction decayed to a fairly constant value, the voltage scan was started, and the zinc peak was recorded. An extrapolation of the base line was taken as the zero. It was possible to use this method of compensation when the zinc concentration was tenfold less than the cadmium (second column of zinc currents in Table II). When the zinc was a hundredfold more dilute than the cadmium, neither of the above methods of compensation could be used. In general, the precision of determination of the second component is considerably less than if that substance were the only reducible species present.

The decrease in sensitivity at high concentrations of both zinc and cadmium was again due to internal resistance drop in the cell, causing a nonlinearity in the rate of voltage change.

Comparison of Results to Theory. Diffusion currents at the spherical electrode have been studied by Frankenthal and Shain (*2*) for the case of a reversible reduction where the products are soluble in the electrode or in the solution. Further conditions to the derivation were that the only mass transfer process operative is diffusion, and that the voltage varies linearly with time. Under these conditions the current-voltage curve is described by the equation (at 25° C.):

$$i = 881 n^{3/2} D^{1/2} A v^{1/2} C \psi \qquad (1)$$

where

i = current, amperes
n = number of electrons involved in electrode reaction
A = area of electrode, sq. cm.
D = diffusion coefficient of reacting species, sq. cm. per second
v = rate of voltage change, volts per second
C = concentration of reacting species, moles per liter.

The final term in Equation 1 is a function of potential, and depends on the experimental parameters

$$\psi = f\left(\frac{1}{r}\left(\frac{D}{nv}\right)^{1/2}\right) \qquad (2)$$

where r is the radius of the electrode in centimeters. If the values of these quantities are known, the last term in Equation 1 may be determined from the data given by Frankenthal and Shain (*2*), and the entire current-voltage curve may be predicted from theory.

Because peak currents measured with the hanging drop electrode are subject to about the same variation with temperature as are polarographic diffusion currents, any comparison of theoretical with experimental results must be carried out at constant temperature. With the cell thermostated at 25° ± 0.1° C., current-voltage curves were obtained for $1.00 \times 10^{-4} M$ solutions of lead, zinc, cadmium, and thallous ions. The experimental results are compared to theory in Table III.

Table III. Theoretical and Experimental Values of Peak Current

Solution	Peak Current: Exptl.[a], μa.	Av.[a] dev., %	Theory, μa.	Deviation, %
Cd^{++}, $1.00 \times 10^{-4} M$ KCl, $0.1 M$	1.49	0.5	1.50	−0.6
Zn^{++}, $1.00 \times 10^{-4} M$ KNO_3, $0.1 M$	1.47	0.5	1.50	−2.0
Pb^{++}, $1.00 \times 10^{-4} M$ KNO_3, $0.1 M$	1.76	0.5	1.76	0.0
Tl^{+}, $1.00 \times 10^{-4} M$ KNO_3, $0.1 M$	0.942	0.7	0.949	−0.7

[a] Average and average deviation of six determinations on same solution.

The values of diffusion coefficient used in these calculations are those at infinite dilution (*4*), and this undoubtedly accounts for the slightly low results.

These data indicate that the concentration of an unknown solution may be determined from theory to within the 1% error level, provided that the cell is thermostated, and that reasonable values of diffusion coefficient are available. Unfortunately, polarographic diffusion coefficients are frequently determined in the presence of maximum suppressors which are not used with the hanging drop electrode.

CONCLUSION

Voltammetry with the hanging drop electrode requires the use of a recorder, whereas in conventional polarography relatively inexpensive manual polarographs are suitable for routine determinations. Furthermore, it is probably somewhat easier to apply compensation in conventional polarography when certain types of mixtures are to be determined. On the other hand, voltammetry with the hanging drop electrode is as reproducible, more rapid, and more sensitive than polarography. These factors, combined with the fact that in most cases concentrations can be calculated from theory with great accuracy, make voltammetry with the hanging drop electrode a very important analytical tool.

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RECEIVED for review May 14, 1956. Accepted July 10, 1956.

Determination of Potassium by a Tetraphenylborate Method

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The use of sodium tetraphenylborate for the determination of potassium is becoming increasingly popular. A method described here for the determination of potassium in the presence of the ammonium ion is superior to the classical chloroplatinate method from the standpoint of accuracy, analysis time, and reagent cost.

NUMEROUS methods for potassium determination may be found in the literature; the chloroplatinate and flame photometric methods are the most widely used. The chloroplatinate method is extremely time-consuming and numerous ions coprecipitate with the potassium and affect the accuracy of the method. The flame photometric method is very rapid and finds excellent application where small amounts of potassium are to be determined. However, the dilutions required for samples high in potassium content may affect the accuracy of the method.

The determination of potassium with sodium tetraphenylborate reagent has become increasingly popular. Gravimetric (*6*), volumetric (*2*, *5*), polarographic (*1*), and conductometric (*4*) procedures utilizing this reagent have been described. The main interest in this investigation was finding a suitable procedure using sodium tetraphenylborate for the determination of potassium in the presence of the ammonium ion.

Rudorff and Zannier (*5*) reported a volumetric procedure for the determination of potassium and ammonium with sodium tetraphenylborate in the presence of each other. The ammonium ion is known to react with tetraphenylborate in the same manner as the potassium ion. Therefore, the ammonium ion was first determined by the formaldehyde method of Marcali and Rieman (*3*). The potassium was then determined by a volumetric procedure using sodium tetraphenylborate to precipitate the potassium. The precipitate was filtered, dissolved in acetone and titrated with a standard silver nitrate solution. The method described below is based on this scheme of analysis, except that a gravimetric rather than volumetric determination is made.

REAGENTS

Sodium Tetraphenylborate Solution. A 1% w./v. solution of sodium tetraphenylborate in 0.01*N* sodium hydroxide is used as the precipitant. Sodium tetraphenylborate powder, a Baker's analyzed reagent, was used. The solution should be prepared fresh each day. However, it was found to keep fairly well for several days in a refrigerator.

Potassium Tetraphenylborate Solution. Potassium tetraphenylborate crystals are obtained by precipitation from a potassium chloride solution similar to that described in this paper. The filtered precipitate is dissolved in acetone and the potassium tetraphenylborate recrystallized from this. A saturated aqueous potassium tetraphenylborate solution is used as the wash solution.

Formaldehyde. A 37% formaldehyde solution purchased from the Mallinckrodt Chemical Co. was used.

Sodium Hydroxide. Reagent grade sodium hydroxide pellets are satisfactory.

PROCEDURE

To an aliquot that contains no more than 45 mg. of potassium and up to 150 mg. of ammonium ion add 130 ml. of formaldehyde solution. Swirl gently to mix well and let stand, well stoppered, for about 5 minutes. Add about 6 grams of pelleted sodium hydroxide and dissolve; this makes the resulting solution about 1*N* with respect to the sodium hydroxide. As soon as the sodium hydroxide has dissolved, heat just to boiling on a hot plate. Remove, slowly add 50 ml. of the 1% sodium tetraphenylborate solution from a pipet, and swirl gently while adding the reagent. Cool to room temperature, swirling several times during the cooling period to ensure adequate mixing. The cooling period may be accelerated by placing the flask in a pan of chilled water. When the solution has reached room temperature, filter through a weighed, medium-porosity, Gooch crucible. Wash well with distilled water saturated with potassium tetraphenylborate. Dry at 110° C. for 1 hour, cool in a desiccator, and reweigh. The gravimetric factor for potassium in the precipitate is 0.10912.

DISCUSSION OF RESULTS

The results of the above method are shown in Table I. The test solutions were prepared by weighing reagent grade potassium chloride into a flask, adding a weighed amount of ammonium chloride, and dissolving in distilled water. In several instances aliquots were taken from a stock solution of potassium chloride and tested. It may be seen from the table that the ratio of potassium to ammonium ions varied from 2:1 to 1:4. The average recovery of potassium by this method was 99%. For the two cases shown, the recovery of potassium by the tetraphenylborate method was closer to theoretical than by the chloroplatinate method. The analysis time by this method is a third that required by the chloroplatinate method.

Table I. Determination of Potassium in Presence of Ammonium Ion

NH_4^+, Mg.	K^+ Weighed, Mg.	K^+ Found, Mg.[a]	
		TPB	CP
3	5.9	5.9	...
22	10.3	10.3	...
40	13.1	13.0	...
60	13.1	12.7	...
40	15.6	15.5	15.0
45	24.4	24.6	...
80	31.2	30.8	30.0
60	31.4	30.6	...
140	32.1	31.6	...
85	43.2	42.3	...

[a] TPB. Tetraphenylborate method. CP Chloroplatinate method.

Potassium is determined regularly in samples containing sodium, potassium, ammonium, chloride, nitrate, sulfate, and phosphate. Suspected high potassium results were attributed to the presence of sulfate and phosphate in the analysis mixture. Table II shows a comparison of the tetraphenylborate and chloroplatinate methods on several samples of this type. In all cases the chloroplatinate results are high. The cation-anion balance for the analysis mixtures was better when the tetraphenylborate

Table II. Comparison of Tetraphenylborate and Chloroplatinate Methods

K^+, %	
TPB	CP
4.79	4.90
5.94	6.17
5.93	6.26
5.87	5.96
4.33	5.93
6.27	6.27
5.89	6.03
7.28	7.59

result was used. This indicated the superiority of the method over the chloroplatinate method.

The volumetric procedure of Rudorff and Zannier (*5*) has been described. The determination of the ammonium ion and potassium in the same aliquot has not been tried; but the method should be most satisfactory, since the formaldehyde determination of ammonium is a very accurate method. Although the alkalinity of the test solution due to the addition of sodium hydroxide should conceivably eliminate any ammonium ion on heating, a large excess of formaldehyde ensures quantitative elimination of the ammonium ion. The ammonium ion is known (*3*) to react quantitatively with formaldehyde to form hexamethylenetetramine. Rudorff and Zannier reported higher than theoretical results if a large excess of formaldehyde were not present or if the solution were only weakly alkaline. The gravimetric procedure was preferred to the volumetric procedure only from the standpoint of the analyst's time. Both procedures require precipitation and filtration. Once the precipitate is filtered, an analyst need only reweigh after the drying period is completed.

A conductometric method using lithium tetraphenylborate to determine potassium in the presence of sodium, magnesium, calcium, strontium, or barium has been developed by Raff and Brötz (*4*). The method is very rapid and possibly could be adapted to the determination of potassium in the presence of the ammonium ion.

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RECEIVED for review March 26, 1956. Accepted July 9, 1956.

Polarographic Determination of Aluminum and Zinc in Magnesium Alloys

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A method for polarographic determination of aluminum in alloy steels has been adapted to permit simultaneous determination of zinc and has been applied to analysis of magnesium-base alloys. An illustration of the effectiveness of this method in studying inverse segregation in magnesium alloys is given.

IN THE course of an investigation of segregation of aluminum and zinc in magnesium alloys, the need arose for a rapid and reasonably accurate method for determining these elements. A gravimetric method for aluminum (*7*) was unsuitable by reason of both the time required and the fact that zinc could be detected in the ignited residue. Gull (*1*) reported a polarographic method for aluminum, lead, zinc, and manganese in magnesium alloys. This method was suitable for zinc determinations but in the case of aluminum there was some difficulty due to masking of the aluminum wave by a hydrogen wave at pH 3 or less. A similar method has been reported by Heller and Zan'ko (*2*, *3*), who also found difficulty with the aluminum determination. Unfortunately, buffer solutions cannot be used to overcome this difficulty (*5*).

Willard and Dean (*9*) have described a method for polarographic determination of aluminum in limestone, iron ores, copper-base alloys, and alloy steels containing 0.1 to 1% of the metal. The present paper deals with the adaptation of the Willard and Dean method to the determination of both aluminum and zinc in magnesium-base alloys.

APPARATUS AND REAGENTS

A Sargent Model XII polarograph was used in this work. No other specialized apparatus was involved.

Pontochrome Violet SW (sodium salt of 5-sulfo-2-hydroxybenzeneazonaphthol), obtained from E. I. du Pont de Nemours & Co., was used in 0.05% aqueous solution. Samples of the same compound (designated Solochrome Violet) can be obtained from Imperial Chemical Industries, Ltd.

STANDARD SOLUTIONS

The standard solutions of aluminum and zinc were prepared by dissolving the reagent grade metals in 5*M* perchloric acid and diluting with water to give each standard a concentration of 10 grams per liter. The magnesium standard was prepared from magnesium perchlorate and made up to contain 20 grams of magnesium per liter.

PROCEDURE

A 0.5-gram sample of the alloy was dissolved by careful addition of 10 ml. of 5*M* perchloric acid and diluted stepwise to give a final concentration of 5 mg. of magnesium in 50 ml. of the solution containing the dye. Neutralization and buffering were carried out as outlined in the original work (*9*) and by Kolthoff and Lingane (*5*, *6*). A portion of the final solution was transferred to a polarographic cell, the dissolved air removed with nitrogen, and the polarogram recorded. The standard solutions were treated in the same way and the aluminum content of the unknown was determined by comparison of the heights of the second waves at −0.5 volt *vs.* S.C.E. Zinc content could be determined by comparison of height of the waves at −1.2 volts *vs.* S.C.E. but in cases where the aluminum-zinc ratio was greater than 1, this wave was too small for accurate measurement. In such cases, zinc was determined from the polarogram of an aliquot of the original solution equivalent to a 0.1-gram sample diluted to 50 ml.

RESULTS AND DISCUSSION

The polarograms show the typical double wave reported by Willard and Dean (*9*), the second wave (−0.5 volt *vs.* S.C.E.) being that used for determination of aluminum. Diffusion currents of a series of standard solutions of aluminum plotted against their concentrations gave a calibration graph identical to that of Willard and Dean. A similar graph can be prepared for zinc, using the height of the third wave at −1.2 volts *vs.* S.C.E. This also applies when zinc is determined on a separate aliquot of the original solution.

The high sample dilution used was made necessary by the limited solubility of the dye. Possible substitute dyes are Super-

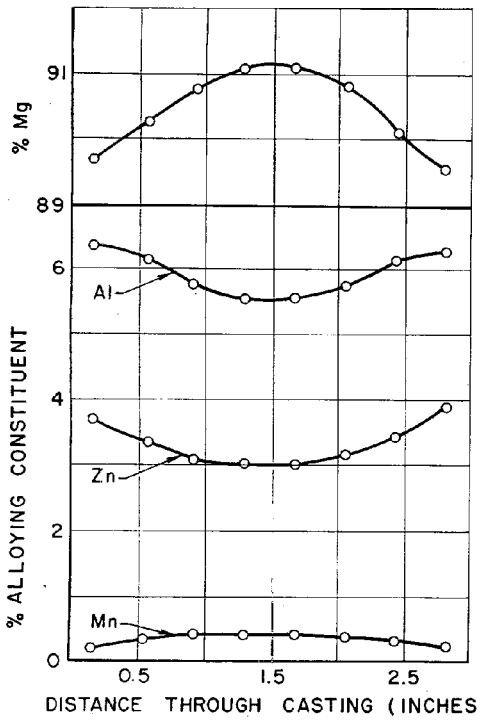

Figure 1. Segregation of major constituents in a magnesium casting

chrome Garnet Y and Pontochrome Blue Black R. The latter is often supplied in the form of a zinc salt and a polarogram of the pure dye should be checked for a zinc wave before proceeding with the determination of this element.

In the course of this work, it was found that manganese can be determined colorimetrically with periodate in an aliquot of the original perchloric acid solution, thus avoiding the necessity of weighing out a separate sample.

The comparative method (graphic measurement and comparison with a standard) was used in all cases in calculating the aluminum or zinc concentration of unknown solutions. Taylor (*8*) has examined the comparative and absolute methods and concluded that the former is more suitable for routine analytical application, as it is not necessary to exercise a rigid control over conditions if standards are run with each series of samples.

Polarographic maxima were not seen in any of the polarograms recorded. Methyl red and bromophenol blue are acting as maximum suppressors as well as indicators in this case. The use of methyl red as a maximum suppressor has been discussed by Kolthoff and Lingane (*4*).

This method was intended for use in the study of segregation of major constituents in magnesium alloys. An illustration of the effectiveness of this method in showing differences in the composition of adjacent samples is given in Figure 1. Drillings were taken from a magnesium casting and analyzed according to the above procedure. In this figure, the results of these analyses are plotted against the position of the sample in the casting. The percentage of magnesium as determined by difference is included and shows a typical inverse segregation effect. Results of the colorimetric manganese determination are also shown.

ACKNOWLEDGMENT

This work was carried out at the Naval Research Establishment of the Defence Research Board of Canada as part of Project Number D12-75-35-02. The permission of the board to publish this work is gratefully acknowledged.

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RECEIVED for review February 27, 1956. Accepted June 25, 1956.

Spectrochemical Analysis of Fabricated Steel with the Rotating Electrode

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A simple, rapid, and accurate procedure for the spectrochemical analysis of steels in acid solutions has been developed. Condensed-spark excitation is used with a rotating graphite electrode. Chromium, copper, manganese, molybdenum, nickel, silicon, and vanadium are determined with a precision within 2 to 4%. The procedure is particularly suited to the needs of the steel consumer, because he cannot control the physical form or metallurgical history of his samples. The steel producer may find the procedure useful for classifying scrap. Extension to the analysis of samples of other metals an dalloys is possible.

CONVENTIONAL spectrochemical methods, in which the sample itself serves as one or both of the electrodes, are often of little use for analyzing fabricated steel. Serious problems arise because the steel consumer is not able to control the physical form or metallurgical history of his sample. The steel producer may encounter similar difficulties in the classification of scrap.

A method that analyzes steels in solution would not suffer these disadvantages. Several methods have been described for introducing metal solutions into the excitation zone. Electrode carbons have been impregnated with steel solutions (*7*), a continuous flow of metal solution has been fed through a capillary

hole drilled in an electrode along its axis (*4*), and porous-cup electrodes (*2*), through which the sample solution seeps, have been used for analyzing bronze in acid solution (*6*). A rotating electrode has been used successfully in these laboratories for determining metals in catalyst solutions (*5*). Investigations of the applicability of this device to the analysis of steel solutions has led to the development of a simple, rapid, and accurate method for determining chromium, copper, manganese, molybdenum, nickel, silicon, and vanadium.

METHOD

Ordinary laboratory glassware and equipment is used for the preparation of samples and standard solutions. The spectrographic equipment (commercially available through Applied Research Laboratories, Glendale, Calif.) includes a grating spectrograph providing a dispersion of 5.2 A. per mm. in the first order, a high-precision source unit (*3*), a Universal arc-spark stand equipped with a rotating-electrode attachment, and a comparator-densitometer.

The rotating-disk apparatus is illustrated in Figure 1. A disk 0.125 ± 0.005 inch thick, cut from a high-purity graphite rod 0.5 inch in diameter, is mounted on a tapered rotating carbon shaft. A stream of air is directed through the excitation zone by positioning a 1/4-inch gas-inlet tube above the optical axis inclined toward the excitation zone. The current of air directs the vapor cloud away from optical surfaces.

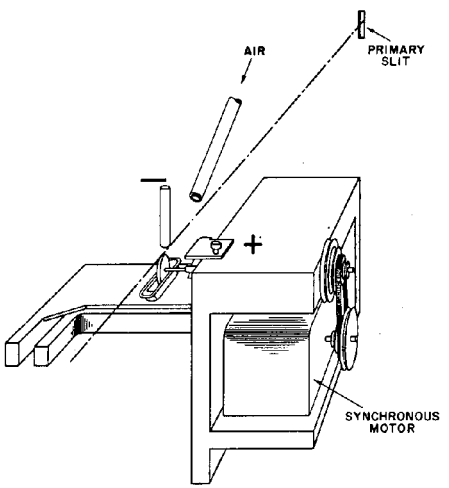

Figure 1. Rotating-disk apparatus

The steel samples are prepared as drillings to obtain material in convenient form for weighing and dissolution; 0.50 ± 0.05 gram of the sample is weighed into a 125-ml. Erlenmeyer flask and treated with acid to dissolve it. No single acid or acid mixture is satisfactory for all steels. If silicon is not to be determined, a usually satisfactory treatment is: 10 ml. of a mixture of 1 volume of 70% perchloric acid plus 2 volumes of 85% phosphoric acid, followed by 15 ml. of a mixture of 1 volume of 70% perchloric acid plus 2 volumes of 80% sulfuric acid (*8*). If silicon is to be determined, 25 ml. of 6*N* nitric acid is often satisfactory.

The particular acid used does not appear to be critical, provided samples and standards are treated alike and solution of the elements to be determined is complete. No attempt has been made to find a dissolution procedure for those steels not soluble in nitric acid but requiring a silicon determination. When solution is complete, the volume is adjusted to 50 ml. Because the iron serves as an internal standard, 10% variation in sample weight, liquid volume, and acid content will not affect the accuracy of the method.

Chemically analyzed steels dissolved in the same manner as the samples serve as standards. Alternatively, synthetic standards may be prepared by dissolving pure metal constituents.

A portion of the prepared sample or standard solution is transferred to a small porcelain combustion boat and placed on the lower electrode clip of the arc-spark stand. The clip is raised until the lower quarter inch of the rotating disk dips into the solution. A fresh disk is used for each exposure. The disk, which is of positive polarity, is rotated at 5 r.p.m. A hemispherically tipped counterelectrode cut from a graphite rod 0.25 inch in diameter is used. The clip holding the counterelectrode is water-cooled.

Ten liters of air per minute are directed through the excitation area. High-voltage condensed-spark excitation is used. Excitation and exposure constants are shown in Table I. Spectrum Analysis No. 1 film is used for recording spectra; film processing, photometry, and calculation of results are carried out in the conventional manner. Spectral lines used and related data are shown in Table II.

Table I. Excitation and Exposure Constants

Added inductance, μh.	360
Current, amperes	2
Analytical gap, mm.	3
Electrode speed, r.p.m.	5
Electrode diameter, inch	0.5
Electrode thickness, inch	0.125
Primary slit width, μ	60
Air flow, liters/min.	10
Pre-exposure period, sec.	20
Exposure period, sec.	60

Table II. Analytical Lines and Related Data

(Internal standard. Fe 3205.4 A.)

Analytical Line, A.	Index[a], %	Range, %	Limit of Detection, %
Cr 2677.2	0.15	0.07 –0.6	0.07
Cr 2822.4	2.3	0.5 –3.5	...
Cu 3274.0	0.045	0.023–0.15	0.023
Mn 2933.1	0.61	0.27 –1.2	0.27
Mo 3170.4	0.25	0.15 –0.7	0.15
Ni 3414.8	0.20	0.1 –0.7	0.10
Ni 3012.0	0.93	0.5 –3.5	...
Si 2881.6	0.32	0.17 –0.4	0.10
V 3184.0	0.28	0.1 –0.5	0.17

[a] Concentration at which intensity of analysis line equals that of standard line.

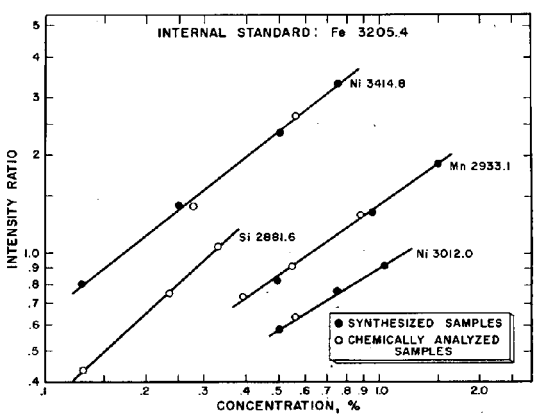

Figure 2. Analytical curves

Table III. Precision of Steel Analysis

Analytical Line, A.	No. of Detns.	Average Concn., %	Coefficient of Variation
Cr 2677.2	32	0.76	2.1
Cr 2822.4	32	4.00	1.8
Cu 2274.0	64	0.087	2.1
Mn 2933.1	64	0.68	1.9
Mo 3170.4	47	0.38	1.7
Ni 3414.8	32	0.44	2.7
Ni 3012.0	32	1.75	1.9
Si 2881.6	15	0.23	4.1
V 3184.0	15	0.23	1.5

DISCUSSION

Precision of the method is shown in Table III. Expressed as coefficient of variation, the precision for all the metals but silicon is within about 2%—that is, the 95% confidence limit of a single analysis is about 4% of the amount present. Two questionable results of 235 determinations were rejected in preparing Table III. Criterion for rejection was a 95% confidence as shown by the statistical Q test (*1*). The error for silicon, although twice that for the other elements, is within acceptable limits.

Good accuracy is indicated by comparative testing of synthetic standards and of steel standards certified by the National Bureau of Standards. The analytical curves for manganese and nickel, shown in Figure 2, indicate good agreement. The slope of the curve for silicon and the precision data indicate that little silicon separates from nitric acid solution; it is probably colloidally dispersed and can be determined accurately.

Sensitivity of the method is shown in Table II. A spectral-line-plus-background intensity 1.5 times that of the adjacent background was considered limiting in arriving at these values. These sensitivities are generally satisfactory for the inspection of fabricated steel parts and for classifying scrap. Increased sensitivity, if desired, can be obtained either by increasing the concentration of the steel samples in the test solution or by using interrupted-arc excitation.

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RECEIVED for review November 14, 1955. Accepted June 28, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 4, 1955.

Determination of Carbon and Hydrogen in Organic Fluorine Compounds

Microcombustion Method for Gases, Liquids, and Solids

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A conventional microcombustion procedure for the determination of carbon and hydrogen was frequently found unsatisfactory for the successive analysis of highly fluorinated organic materials. A modified combustion tube filling, maintained at 900° C. and containing two sections of magnesium oxide separated by a section of copper oxide, ensures complete oxidation of the sample and removal of fluorine from the combustion products. The precision found was somewhat better for hydrogen and somewhat poorer for carbon than that for the nonfluorinated samples using a conventional procedure. Because the apparatus includes a system for measuring and transferring gaseous samples to the combustion tube, it can thus be used for samples ranging from gases to solids.

VARIOUS workers have shown that conventional procedures for the determination of carbon and hydrogen in organic fluorine compounds are often not satisfactory because the fluorine-containing combustion products react with the silica combustion tube to form silicon tetrafluoride. This gas passes into the absorption train and is retained, causing high results (*2*, *15*). Throckmorton and Hutton (*15*) recently reviewed a number of combustion procedures for this determination and subsequently used magnesium oxide placed in the sample end of the microcombustion tube for removing fluorine from the combustion products.

Because fluorinated organic materials may contain little or no hydrogen, their hydrogen content is a sensitive measure of the extent of fluorination and it must be determined accurately. It is well known that lead peroxide, frequently used in combustion tube fillings to absorb oxides of nitrogen, is partially hydrated. Close control of the amounts of water produced by the sample and the volumes of carrier gases passed over the lead peroxide is necessary to maintain a steady state and to obtain quantitative transport of water to the absorption train (*5*, *7*, *9*). Thus, samples containing little or no hydrogen produce little or no water and dehydrate the lead peroxide, causing high hydrogen results (*15*). Subsequent analyses of compounds containing more hydrogen give low results for hydrogen, because the dehydrated lead peroxide absorbs some of the water.

The physical properties of highly fluorinated organic materials are such that many of them are gases. For this reason a general method for the analysis of these compounds should include provision for measurement and transfer of gaseous samples to the combustion tube.

The procedure described here makes use of magnesium oxide as part of the combustion tube filling as suggested by Throckmorton and Hutton. However, with the types of compounds analyzed and the apparatus and procedure used, it was found necessary to place the magnesium oxide differently to ensure complete retention of fluorine. The lead peroxide section in the combustion tube filling used by Throckmorton and Hutton was omitted, as they suggested, to avoid the hydration problems mentioned above and thus to achieve greater accuracy for hydrogen

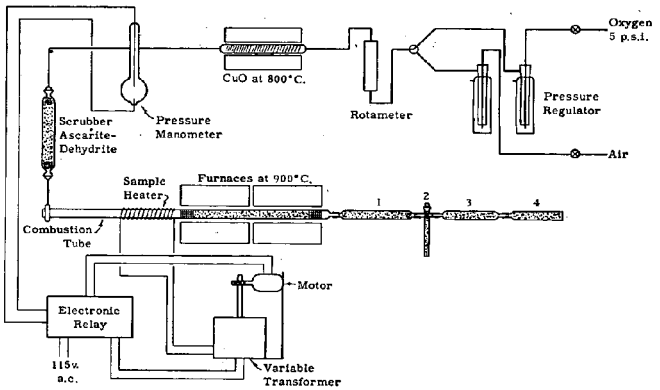

Figure 1. Micro carbon and hydrogen apparatus for organic fluorine compounds

Absorber train
1. Dehydrite absorber
2. Manganese dioxide absorber
3. Ascarite absorber
4. Guard tube (Ascarite-Dehydrite)

determinations. When samples containing nitrogen were analyzed, an external absorber containing manganese dioxide, as recommended by Belcher and Ingram (*3*), was used.

A gas measuring system was constructed which enables small volumes of gases to be measured directly from pressurized metal cylinders and quantitatively transferred to the combustion tube.

A number of highly fluorinated compounds ranging from gases to the solid polymer, Teflon, were analyzed.

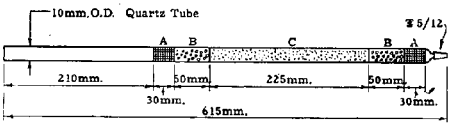

Figure 2. Tube filling

A. Rolled silver gauze (20 mesh)
B. Magnesium oxide (8 to 20 mesh)
C. Copper oxide (wire form)

EXPERIMENTAL

Apparatus. The apparatus used (Figure 1) was a semiautomatically controlled unit similar to that described by Fischer (*7*). The combustion tube filling (Figure 2) contained a 225-mm. center section of wire-form copper oxide flanked on each side by 50 mm. of 8- to 20-mesh magnesium oxide prepared according to Throckmorton and Hutton and 30 mm. of 20-mesh rolled silver gauze. The filled section of the quartz combustion tube was maintained at about 900° C. The combustion gas, either air or oxygen, was metered with micro flowmeters (Technical Equipment Co., Berkeley 10, Calif.) and purified by passing over copper oxide at 800° C. and through an absorber which contained the conventional absorbents, Ascarite for carbon dioxide and Dehydrite for water. The absorbers were Pregl type, equipped with standard taper joints similar to those described by Fischer. An external absorber containing manganese dioxide to absorb oxides of nitrogen (*3*), placed between the water and carbon dioxide absorbers, was used when analyzing compounds containing nitrogen.

Gas Measuring System. The gas measuring system, shown schematically in Figure 3, consists of a panel-mounted 1-ml. gas buret provided with an air-operated mercury lift. The buret was made by modification of a 1-ml. titration buret and was fitted with a four-way stopcock whose bore permits any two adjacent arms to be connected (Catalogue No. 4890, Eck and Krebs, New York, N. Y.). It was immersed in an air-stirred water jacket to stabilize its temperature. The buret is calibrated through the stopcock because the gas in the stopcock bore is transferred to the combustion tube. Although a 1-ml. buret was used in this apparatus, use of a 2-ml. buret would have enabled a more suitable sample size to be measured without refilling.

A combustion tube closure is fitted with a platinum tube 2.5 mm. in diameter which extends into the first silver gauze section of the tube packing. The platinum tube is connected to the buret with 22-gage stainless steel hypodermic tubing (Superior Tube Co., Norristown, Pa.). A vacuum pump complete with cold traps and a manometer is connected to one arm of the buret.

All metal to glass joints were made using spherical joints sealed together with sealing wax. Stainless steel spherical joints (Whitey's Tool and Die Shop, Oakland, Calif.) were silver soldered to the ends of the metal tubing. Soft solder is not suitable because the heat applied to melt the sealing wax may also melt soft solder. The metals used are not considered important except that the sample injector tube must be resistant to high temperatures and oxidation, and brass or copper must not be used in the mercury lift system.

A micro pressure vessel, shown in Figure 4, having approximately 0.2-ml. volume, was used to transfer small increments of gas into the buret when sampling from pressurized metal cylinders. The valves were $^1/_8$-inch Teflon-packed stainless steel needle valves (No. 321, Hoke Inc., Englewood, N. J.) which were shortened on the needle end to remove the enlarged section of the bore. After assembly the threaded nut, made from $^3/_4$-inch hexagonal brass, was soft soldered to the valves to ensure a positive vacuum seal. The vessel was pressure tested. Brass bellows valves (No. 431, Hoke, Inc.) were used originally in place of the recommended valves but were found to be rapidly "pin-holed" by accidental contact with mercury. Small stainless steel hypodermic tubing (22 gage) connects the bomb to the buret to minimize volume and to provide mechanical flexibility.

Materials Analyzed. The acetanilide used was NBS microanalytical standard sample number 141. The *n*-heptane was a pure material suitable for use as a combustion standard. The isobutane was 99.9 mole % pure (Phillips Petroleum Co., Research Grade). All other materials were commercial grades and were not further purified.

To aid in estimating their purity, the fluorine contents of commercial materials were determined in some cases by decomposition in a sodium peroxide bomb followed by steam distillation in an apparatus similar to that of Huckabay, Welch, and Metler (*8*), and photometric titration with thorium nitrate using Chrome Azurol S as indicator.

Procedure for Liquids and Solids. The procedure used was similar to that described by Fischer (*7*), except that gas flow rates of 8 to 10 ml. per minute for both air and oxygen were used with a total combustion and sweeping time of 50 minutes. With highly fluorinated materials the sample sizes were adjusted to contain about 5 mg. of carbon. The samples were combusted in oxygen rather than air because no explosion hazard exists with these compounds.

Solid samples were weighed directly in platinum boats. Liquid samples were drawn into empty quartz capillaries by heating and cooling the capillary with the tip in the sample. The capillary was centrifuged to remove the sample from the tip, and was then sealed with a torch. Just before combustion about 3 mm. of the tip was cut off and both parts of the capillary were placed in a platinum tray and inserted into the combustion tube. For very volatile samples it is necessary to crush the sealed capillary inside the combustion tube. (The authors use a technique in which the sealed capillary is placed between two quartz rods 20 mm. in

length and 1 mm. smaller in diameter than the inside diameter of the combustion tube. The capillary has a curved tip which is scored about 3 mm. from its end. These items are placed in the combustion tube and pushed lightly against the tube packing. The combustion tube in the vicinity of the capillary is cooled with solid carbon dioxide and the outer quartz rod is pushed with a rod until the capillary breaks. The combustion tube is then closed immediately, the solid carbon dioxide is removed, and this part of the combustion tube is allowed to come to room temperature. Care must be taken to avoid carbon dioxide from the outside from entering the opened combustion tube.) The capillary was finally heated to red heat. If carbonaceous residues were visible at the end of vaporization the outside of the sample section of the combustion tube was cooled with compressed air to draw oxygen into the capillary, which was then reheated. This technique avoids any necessity for using a combustion aid such as potassium chlorate in the sample capillary (*12*). Quartz capillaries are necessary because glass melts at the temperatures used and carbonaceous material may be trapped in the melt.

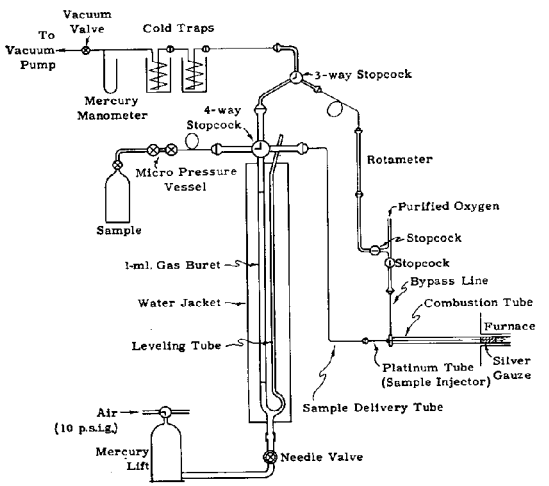

Figure 3. Gas measuring unit

Procedure for Gases. The micro pressure vessel is connected between the metal cylinder or other sample container and the gas buret (Figure 3), using appropriate adapters for connection to the sample container. The buret is filled with mercury to the stopcock. The sample connecting lines, micro pressure vessel, and connecting lines to the sample container are evacuated and the system is tested for leaks by shutting off the vacuum system and observing the manometer for 1 minute or so. The three-way stopcock is then turned counterclockwise to a closed position. The needle valve on the micro pressure vessel nearest the buret is closed, the valve on the sample container is opened to admit sample to the vessel, and the other valve on the micro pressure vessel is then closed. The mercury level in the buret is lowered and the micro pressure vessel is then opened to the buret to allow the sample in it to flow into the buret.

If sufficient sample has not been transferred the micro pressure vessel is refilled and its contents are again transferred to the buret. (*Caution!*) One or the other of the two valves on the micro pressure vessel must always be kept closed, except when evacuating, in order to avoid hazards resulting from release of a large volume of pressurized gas into the glass parts of the apparatus. With the buret stopcock open towards the micro pressure vessel, the sample is adjusted to atmospheric pressure and the volume, atmospheric pressure, and water jacket temperature are recorded.

Weighed absorbers are attached to the combustion tube as in the procedure for liquids and solids and oxygen is passed directly through the bypass line (Figure 3) and into the combustion tube at a rate of 10 ml. per minute. The buret stopcock is turned counterclockwise to connect the buret to the combustion tube and the sample is slowly pushed into the combustion tube at a rate of about 0.2 ml. per minute by means of the mercury lift. When the buret is just filled with mercury, the buret stopcock is turned one quarter turn counterclockwise so that the sample delivery tube can be purged with oxygen and the stopcocks in the oxygen and oxygen bypass lines are adjusted so that the oxygen flow is equally divided between the two lines to complete the transfer of sample in the presence of excess oxygen. If additional sample is desired the above procedure is repeated (including reevacuation) after oxygen has been passed through the sample delivery tube for 5 minutes. The absorbers are removed for weighing 50 minutes from the time the sample was introduced into the combustion tube.

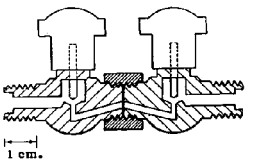

Figure 4. Micro pressure vessel

Calculations. The usual calculations are made for expressing the carbon and hydrogen content of liquids and solids on a weight basis. For gases, the carbon and hydrogen content may be expressed as the weight per unit volume of sample or, as reported by Marion and Ledingham (*10*) for hydrocarbons, as the ratio of carbon to hydrogen. To obtain results on a weight per cent basis, the gas density must be determined.

BEHAVIOR OF CONVENTIONAL TUBE FILLING

The behavior of a conventional microcombustion tube filling (*12*), which consisted of metallic silver and copper oxide maintained at 900° C. and lead peroxide at 195° C. was demonstrated by the analysis of a few perfluorinated oils. The results, made with a new, freshly filled, combustion tube, are shown in Table I in the order in which they were made. The results for some of these same materials, obtained with the recommended tube filling, are also shown in Table II. These data in Table I show that even the first combustion gave somewhat high results and that by the fifth determination the values were markedly high. The subsequent analysis of a fluorine-free standard acetanilide, gave low results, probably because the lead peroxide section of the tube packing was not in an equilibrium state.

DEVELOPMENT OF SPECIAL TUBE FILLING

The first tests were made using a combustion tube filling similar to that used by Throckmorton and Hutton (*15*) in which a 35- to 40-mm. section of magnesium oxide was placed in the hot zone of the tube just ahead of the copper oxide. As they suggested, the lead peroxide section was omitted to increase the accuracy of the hydrogen results. The performance of the apparatus and combustion tube was verified by combustions of *n*-heptane. A number of combustions of organic fluorine compounds were then made. The first four materials, which contained a total of about 60 mg. of fluorine, showed essentially correct results. Subsequent analyses gave correct hydrogen results but high carbon values. Because incomplete retention of fluorine in the combustion tube would result in high carbon values, the Ascarite from the carbon dioxide absorber was analyzed for fluoride as described above. Calculations based on the amount of fluoride found and the assumption that silicon tetrafluoride was the combustion product showed that the weight of silcon tetrafluoride found in the carbon dioxide absorber was approximately equal to the total amount by which the carbon values were high. Throckmorton and Hutton were able to retain larger amounts of fluorine, probably because their combustion tube filling contained lead peroxide. Both lead peroxide and lead sesquioxide have been used in combustion tubes for absorbing fluorine (*11, 14*). Clark and Rees (*4*) used lead oxide at 700° C. to aid in combustion of organic fluorine compounds.

Consideration of the results obtained with this experimental tube packing suggested a possible explanation for its failure to remove all of the fluorine from the combustion products. In the above tube the section of magnesium oxide added for this purpose precedes the section of copper oxide used to ensure complete oxi-

Table I. Microdetermination of Carbon and Hydrogen in Organic Fluorine Compounds Using Conventional Tube Filling[a]

(Determinations made in sequence shown, starting with fresh combustion tube for No. 1)

Analysis No.	Material[b]	Carbon, Wt. % Present[c]	Carbon, Wt. % Found	Hydrogen, Wt. % Present[c]	Hydrogen, Wt. % Found
1	FCX-412, Cut 4	25.0	25.6	0.13	0.93
2			25.7		1.19
3	FCX-412, Cut 7	[d]	25.0	[d]	0.83
4			24.9		
5	FCX-412, Cut 4	25.0	29.0	0.13	1.13
6	FCX-412, Cut 1	25.1	44.0	0.03	4.36
7			44.5		3.47
8	Acetanilide (NBS)	71.1	66.6	6.70	6.48
9			67.8		6.19

[a] (*13*).
[b] FCX-412 perfluorinated oil obtained from E. I. du Pont de Nemours & Co., Inc.
[c] From data in Table II, determined with special tube filling.
[d] Not determined.

Table II. Carbon and Hydrogen Analyses of Organic Fluorine Compounds Using Special Tube Filling Containing Magnesium Oxide

Material	Carbon, Wt. % Calcd.	Carbon, Wt. % Found	Hydrogen, Wt. % Calcd.	Hydrogen, Wt. % Found	Fluorine, Wt. % Calcd.	Fluorine, Wt. % Found[a]	Sum of C, H, F Found, Wt. %
n-Heptane	83.90	83.88	16.10	16.12	..	..	100.0
		83.80		16.08	..	..	99.9
Acetanilide (NBS)	71.09	71.20	6.70	6.75	..	..	..
		71.24		6.81	..	..	..
		71.14		6.76	..	..	..
		71.24		6.74	..	..	..
Perfluorodimethylcyclohexane	24.02	24.13[b]	0.00	0.12[b]	76.0	76.3	100.6
p-Fluorobenzoic acid	60.00	60.27	3.60	3.76	13.56	12.7	..
		60.51		3.71			
Benzotrifluoride	57.53	57.81	3.45	3.65	39.01	39.4	100.9
		57.89		3.63			
p-Fluorochlorobenzene	55.20	55.56	3.09	3.11	14.55	14.5	..
		55.70		3.35			
Perfluorinated oil[c] FCX-412, Cut No. 1	...	25.08	0	0.02	..	75.2	100.3
		25.16		0.04			
Perfluorinated oil[c] FCX-412, Cut No. 4	..	25.09	0	0.12	..	75.0	100.1
		24.95		0.14			
Teflon[c] (Polyperfluoroethylene)	24.0	23.80	0	0.07	76.0	77.1	100.9
		23.84		0.02			

[a] Average of duplicates.
[b] Average of 13 determinations.
[c] E. I. du Pont de Nemours & Co., Inc.

dation of the sample. Thus, it was possible that all of the fluorine in the samples had not been converted to a form removable by the magnesium oxide until after the combustion products had gone past the magnesium oxide. To test this hypothesis, a second tube packing was prepared in which a section of magnesium oxide was placed on each side of the copper oxide. The dimensions and materials used in this tube filling are shown in Figure 2. Because magnesium hydroxide is stable to about 350° C. and magnesium carbonate to about 480° C. (*6*), it was necessary to maintain the magnesium oxide at a higher temperature to prevent retention of carbon dioxide and water. For convenience, the entire combustion tube filling was heated to 900° C. The normal gas flow rate of 8 to 10 ml. per minute was used. Tests with water, calcium carbonate, and *n*-heptane showed that it was necessary to use a combustion cycle of 50 minutes to obtain quantitative transport of water and carbon dioxide through the combustion tube.

Applicability. This special tube filling and recommended procedure were tested by analysis of a number of organic fluorine compounds. These materials were not known to be pure; therefore, their fluorine contents were also determined. Table II shows that the results obtained were close to the theoretical values and that the sum of the carbon, hydrogen, and fluorine found, when other elements were absent, was correct within the limit of accuracy of the fluorine determination (normally ±1%). No systematic error is apparent in these results.

The retention of fluorine by the tube packing was tested, after combustion of a quantity of fluorine-containing materials equivalent to 300 mg. of fluorine, by analysis of the absorbers. Less than 0.1 mg. of fluoride was detected. The maximum capacity of the tube filling for fluorine was not determined.

Nonfluorinated materials were also successfully analyzed in the special tube filling as shown by the results with *n*-heptane and acetanilide (Table II).

Precision. Calculations of the pooled standard deviation (*16*) of the carbon and hydrogen results shown in Table II were made; similar calculations were also made for carbon and hydrogen microdeterminations on routine nonfluorinated samples using the conventional tube filling described above under the same operating conditions. These values are given in Table III. The difference between these standard deviations are significant at the 95% confidence level. The precision of carbon determinations on organic fluorine compounds using the special tube filling was somewhat poorer and the precision of hydrogen determinations somewhat better than that found for nonfluorinated samples using a conventional apparatus. The reason for the somewhat poorer precision found for carbon with organic fluorine compounds is not known; the limited number of tests made with the special tube filling on nonfluorinated materials indicates a precision for carbon equivalent to the conventional tube filling (Table II). The improved precision for hydrogen with the recommended tube filling is attributed to the removal of the lead peroxide from the tube filling.

COMBUSTION OF GASES

To test the procedure as applied to the analysis of gaseous organic fluorine materials, a number of combustions using isobutane were made.

In the initial experiments with isobutane, incomplete combustions, as evidenced by low results, were obtained when air was used as the combustion gas. The recommended procedure in which the sample was pushed through the injector tube with oxygen while an equal amount of oxygen was flowing directly into the combustion tube was found necessary to obtain satisfactory results. (Although the procedure directs transfer of the gas from the buret before the oxygen flow is divided, the volume of the tubing between the buret and the combustion tube is such that little, if any, sample leaves the tubing before the oxygen flow is split.) No difficulty was experienced with explosions or flashing of this flammable gas because the small diameter platinum injection tube discharges the sample directly into the hot silver gauze at the beginning of the tube filling. Commercial Freon 114 ($CClF_2CClF_2$, Du Pont) and Genetron 150 (CH_2F_2, Allied Chemical and Dye Corp.) were also analyzed, using oxygen during combustion. Reasonable values were obtained. The data found

Table III. Pooled Standard Deviation of Micro Carbon and Hydrogen Procedures

Procedure	Pooled Standard Deviation (*16*), % Carbon	Hydrogen
Conventional[a]	0.10	0.12
Special[b]	0.17	0.07

[a] Using nonfluorinated compounds. Tube filling as described in section on behavior of conventional combustion tube.
[b] Using organic fluorine compounds; calculated from data in Table II.

Table IV. Micro Determination of Carbon and Hydrogen in Gaseous Materials

Material	Obsd. Volume, Ml.	Pressure, Mm. Hg	Temperature, ° C.	Carbon, Wt. % Calcd.	Carbon, Wt. % Found	Hydrogen, Wt. % Calcd.	Hydrogen, Wt. % Found
Isobutane[a]	2.199	768	24.7	82.66	83.14	17.34	17.51
	2.208	771	23.0		82.92		17.44
	2.252	770	25.7		83.10		17.45
				Av.	83.05	Av.	17.47
Freon 114[b,c]	2.238	763.5	25.0	14.05	14.09	0.00	0.65[c]
	2.321	768.5	23.0		13.90		0.05
	2.175	767.5	24.0		13.88		0.05
				Av.	13.96	Av.	0.05
Genetron 150[b]	2.175	770	23.2	37.52	37.05	3.15	3.66
	2.193	769	23.7		36.93		3.38
	2.138	768.5	23.5		37.18		3.30
				Av.	37.05	Av.	3.45

[a] Carbon and hydrogen values calculated using density of 6.482 cu. ft./lb. at 1 atm. and 70° F. (*13*). Corrections for deviations from perfect gas law negligible for these small temperature differences.
[b] Corrections for deviations from perfect gas law (*1*) made in calculation of weight per cent carbon and hydrogen.
[c] High value may be due to moisture in gas buret; excluded from average.

for these compounds and for isobutane are shown in Table IV. The sample of Freon 114 tested appears to be pure, while the carbon and hydrogen values and the ratio of carbon to hydrogen found in the Genetron 150 sample indicate that it contains an impurity with a lower fluorine content.

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RECEIVED for review January 18, 1956. Accepted June 16, 1956.

Precipitation of Actinium Oxalate from Homogeneous Solution

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Actinium separated by ion exchange from neutron-irradiated radium was found to contain nonradioactive impurities, mostly iron and aluminum. These impurities interfered with the reduction of actinium to the metal. A method is described for purifying actinium by oxalate precipitation from homogeneous solution. Yields of 97% or more were obtained. The solubility of actinium oxalate in 0.1*N* nitric acid–0.5*N* oxalic acid solution was 0.024 mg. per ml.

ACTINIUM can be synthesized by the neutron irradiation of radium and separated from the radium by ion exchange on Dowex-50 resin (*5*). However, nonradioactive impurities, chiefly iron and aluminum, are introduced from the resin. These impurities, which may constitute an appreciable percentage of the product, interfere with the reduction of actinium to the metal (*14*).

Oxalate precipitation from weakly acid solution has been used to separate lanthanum and the rare earths from iron, aluminum, and many other elements (*15*). Several investigators (*4*) have coprecipitated tracer quantities of actinium with lanthanum oxalate, but only a few micrograms of actinium has been precipitated as oxalate without lanthanum carrier (*2*). In this paper the carrier-free precipitation of macro quantities of actinium oxalate is discussed. Precipitation from homogeneous solution (*3*, *16*) was effected by the hydrolysis of dimethyl oxalate to produce oxalate ions uniformly within the solution.

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PROCEDURE

The actinium fraction obtained from the ion exchange column was evaporated in a small filter-beaker (*11*) until a precipitate began to form. Sufficient water was added to dissolve the precipitate. The pH was adjusted to 1 to 2 by the dropwise addition of 3*N* ammonium hydroxide until 1 drop formed a hydroxide precipitate. Then 1 to 20 nitric acid was added dropwise until the hydroxide dissolved. Water was added until the actinium concentration was 0.5 curie (about 7 mg.) per ml., followed by the addition of three times the stoichiometric amount of dimethyl oxalate dissolved in the minimum quantity of methanol. (The dimethyl oxalate was recrystallized from methanol, prior to its use, to remove possible oxalic acid impurity.) The solution was stirred and heated at 60° to 70° C. The dimethyl oxalate slowly hydrolyzed and, after a few minutes, the solution became turbid with actinium oxalate. The mixture was digested with stirring at 60° to 70° C. for 30 minutes and then stirred at room temperature for an additional 90 minutes. The oxalate was filtered and washed with 1% oxalic acid solution.

The oxalate was decomposed with a few drops of red fuming nitric acid. Occasionally actinium nitrate was produced. It dissolved upon the addition of a few drops of water and, thus, could be differentiated from the oxalate. The nitrate solution was evaporated to dryness to remove the excess nitric acid. The resulting actinium nitrate was dissolved in water and the actinium concentration again adjusted to 0.5 curie per ml. A second oxalate precipitation was made by the procedure described above. The actinium oxalate obtained after the second precipitation was also converted to the nitrate.

The nitrate was dissolved in the minimum quantity of water, transferred to a small, weighed platinum crucible, and carefully evaporated to dryness under an infrared lamp to remove excess

nitric acid. The nitrate was dissolved in 1 to 2 ml. of water. To this solution was added an excess of 5% oxalic acid. The mixture was carefully evaporated to dryness under an infrared lamp. The residue was dried at 200° C. in a small muffle furnace for 30 minutes and then ignited at 900° C. for 90 minutes. The platinum crucible was cooled to room temperature and weighed, and the weight of actinium oxide was calculated.

As actinium is a highly radioactive and biologically dangerous material, it was necessary to carry out this work in a well ventilated dry box. The glassware, which became brown and crazed because of the intense radiation, was handled through glove ports and behind 2 inches of lead shielding.

RESULTS AND DISCUSSION

Actinum nitrate was converted to oxalate prior to ignition to the oxide, to prevent spattering during ignition. Lanthanum nitrate, for example, melts prior to decomposition and some lanthanum can be lost through spattering upon conversion of the nitrate to the oxide.

When precipitated from homogeneous solution, the actinium oxalate was a very dense white crystalline material. The oxide obtained upon the ignition of the purified oxalate was also a white compound, which glowed with a bright white luminescence visible even in a lighted room.

The filtrates from the oxalate precipitations were analyzed radiochemically for actinium (*9*). The method consisted of a double thorium iodate precipitation in 5 to 6*N* nitric acid with thorium carrier to remove thorium-227, followed by a double barium nitrate precipitation in 80% nitric acid with barium carrier to remove radium-223. Aliquots of the solution containing the freshly purified actinium were mounted on stainless steel disks and alpha-counted a day or more after purification. The counts were corrected for the growth of actinium decay products (*8*). The actinium concentration in the filtrates was calculated from these corrected alpha counts, assuming a 22.0-year half life (*7*).

The results of the analyses of the filtrates from two actinium purifications are shown in Table I. The actinium originally in sample 1 (27.5 mg.) was determined by a calorimetric method (*12*). No original calorimetric measurement was made on sample 2. The actinium recovered as oxide in each case was determined gravimetrically. The results indicate a high recovery of actinium by the oxalate precipitation method. The loss of actinium in the first oxalate filtrate was several times greater than that in the second filtrate. The high concentration of impurity present during the first precipitation apparently increased the solubility of actinium oxalate.

Table I. Purification of Actinium by the Oxalate Method

Fraction	Actinium Mg.	Actinium %	Iron Mg.	Iron %
		Sample 1		
Actinium oxide	26.5	96.5	Nil	...
First oxalate filtrate	0.4	1.5	0.60	97
Second oxalate filtrate	0.06	0.2	0.02	3
Total	27.0	98	0.62	
Original	27.5		(Fe/Ac = 1/10)[a]	
		Sample 2		
Actinium oxide	43.5	98.0	Nil	...
First oxalate filtrate	0.8	1.8	4.10	99
Second oxalate filtrate	0.1	0.2	0.03	1
Total	44.4		4.13	
Original			(Fe/Ac = 4/10)[a]	

[a] Mole ratio.

Polarographic determination of iron in the filtrates (*10*) showed that 97% or more of the iron was removed by a single oxalate precipitation (Table I). The mole ratio of iron to actinium originally in the first sample was 1 to 10 and in the second, 4 to 10.

In addition to iron, aluminum was detected by spot tests (*1*) in the filtrates from the first oxalate precipitations but not in those from the second. Addition of barium gave a test for sulfate in the first oxalate filtrates. The sulfate probably resulted from the radiation decomposition of the ion exchange resin.

The solubility of actinium oxalate can be estimated from the results of the analyses of the second oxalate filtrates. The solution had a pH of 1.2 and was approximately 0.5*N* oxalic acid. For the two separate actinium purifications the second oxalate filtrates contained 0.0149 and 0.0158 mg. of actinium per ml., respectively. The solubility of actinium oxalate calculated on the basis of the anhydrous compound was 0.024 mg. per ml. This value compares favorably with solubility values reported by Sarver and Brinton (*18*) for lanthanum oxalate in similar solutions. For example, as shown in Table II, the solubility of lanthanum oxalate in 1*N* hydrochloric acid–0.5*N* oxalic acid was reported as 0.06 mg. per ml. In 0.1*N* mineral acid–0.5*N* oxalic acid, the solubility of lanthanum oxalate would be less than this value. It is probable that the solubilities of actinium and lanthanum oxalates do not differ greatly.

Other estimated values for the solubility of actinium oxalate are shown in Table II. Wever (*6*) estimated the solubility of actinium oxalate in 0.1*N* hydrochloric acid by tracer distribution studies with rate earth oxalates. The value of 0.5 mg. per ml. indicated that actinium oxalate was more soluble than lanthanum oxalate (0.21 mg. per ml.). In either case, the addition of oxalic acid decreased the solubility.

Table II. Solubilities of Actinium and Lanthanum Oxalates

Solvent	$Ac_2(C_2O_4)_3$, Mg./Ml.	$La_2(C_2O_4)_3$, Mg./Ml.
0.1*N* HNO_3, 0.5*N* $H_2C_2O_4$	0.024	...
1*N* HCl, 0.5*N* $H_2C_2O_4$	...	0.06 (13)
0.1*N* HCl	0.5 (6)	0.21 (13)
0.05*N* HCl, 0.5*N* $(NH_4)_2C_2O_4$	<0.2 (2)	....
0.03*N* HCl, 0.7*N* $H_2C_2O_4$	>0.14 (2)	....

In the preparation of microgram quantities of actinium oxalate for x-ray samples, Fried, Hagemann, and Zachariasen (*2*) obtained a white dense precipitate when 50 μl. of 1*N* ammonium oxalate solution was added to 10 γ of actinium in an equal volume of 0.1*N* hydrochloric acid, but no visible precipitate when 100 μl. of saturated oxalic acid solution was added to 10 γ of actinium in 50 μl. of 0.1*N* hydrochloric acid. The latter case would indicate a rather high solubility for actinium oxalate, approximately one order of magnitude higher than the solubility found in the present work. However, in the precipitation of very small quantities of lanthanum and rare earth oxalates from mineral acid solutions there is a tendency toward either supersaturation or microcrystalline formation. If this is also the case in the precipitation of microgram quantities of actinium oxalate, a solubility higher than the actual solubility would be observed. This possible source of error was eliminated in the present work by the precipitation of actinium oxalate in macro quantity from homogeneous solution.

ACKNOWLEDGMENT

The authors wish to thank Carlyle E. Shoemaker and Kenneth Jordan for the polarographic and calorimetric analyses.

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RECEIVED for review February 15, 1956. Accepted July 25, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956. Mound Laboratory is operated by Monsanto Chemical Co. for the U. S. Atomic Energy Commission under Contract No. AT-33-1-GEN-53.

Radiochemical Determination of Phosphorus-32

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A method for the radiochemical determination of phosphorus-32 in reactor cooling water is based on solvent extraction of phosphorus as molybdophosphoric acid. After sorption of radioarsenic on cupric sulfide and exclusion of radiosilicon by extraction from high acid concentration, radiochemically pure phosphorus-32 is extracted with a 10% solution of 1-butanol in diethyl ether. The method is rapid, accurate, and reproducible; 86.6% of the phosphorus-32 is recovered by a single extraction.

A METHOD was required for the separation and determination of phosphorus-32 from reactor effluent water, a solution containing mixed activation products and fission products. Phosphorus-32 is a pure beta emitter which decays with a 14.3-day half life. Radiophosphorus contributes about 0.4% of the gross beta activity of this waste stream, the principal constituents of which are arsenic-76, copper-64, manganese-56, silicon-31, and sodium-24 (*2*). Requirements stipulated that analysis should be completed within 8 hours after sampling.

Precipitation of benzidine phosphate or bismuth phosphate as recommended by Hahn and Anderson (*1*) for the radiochemical determination of phosphorus-32 was not feasible, because of coprecipitation of contaminants. The classical ammonium molybdophosphate precipitation likewise proved unsatisfactory, owing to considerable contamination in the final product.

Formation of molybdophosphoric acid and subsequent reduction to molybdenum blue have been applied for the colorimetric determination of phosphate ion in water and other materials. Solvent extraction of molybdophosphoric acid with 1-butanol eliminates all interferences except arsenate, silicate, and germanate (*4*). The interference from germanate ions is of no consequence, as no significant amounts of the radioisotopes of germanium are present in reactor effluent water. Radioactive silicon-31 ($T_{1/2}$ = 2.6 hours) and arsenic-76 ($T_{1/2}$ = 26.8 hours), both beta-emitters, are present. The specific activities of these two isotopes are 10 to 100 times greater than phosphorus-32. Measures are therefore required to remove these interferences. The low distribution coefficient of molybdosilicic acid into 1-butanol from an aqueous medium greater than 1*N* in sulfuric acid has been shown (*4*). By extraction of molybdophosphoric acid from 1.3*N* sulfuric acid, silicon isotopes were easily eliminated. Removal of arsenic from phosphoric acid solutions by sorption on stannous sulfide was reported by Shirasaki and Muroya (*3*). Because of immediate unavailability of stannous sulfide, a search of available metallic sulfides indicated that the properties of cupric sulfide were compatible with the process, and it was selected as the sorption agent.

EXPERIMENTAL

A sorption bed was prepared by placing an aqueous slurry of powdered cupric sulfide over a mat of glass wool and asbestos fibers. The bed was contained in a section of 30-mm. borosilicate glass tubing which was reduced at the outlet to a 2-mm. stopcock. The liquid was removed by suction, care being taken to keep the liquid level above the level of the bed. The bed was then washed with water until soluble copper salts were no longer visible in the column effluent. A cupric sulfide bed, prepared from either freshly precipitated or commercially available material (Baker and Adamson, Lot 14R), was found to remove radioarsenic from 0.05*N* hydrochloric acid solution. It was necessary to maintain arsenic in the arsenite form to assure its removal. Any arsenate ion was therefore reduced with sodium thiosulfate preliminary to sorption on the bed. The removal efficiency of radioarsenic from reactor effluent and spiked tap water samples was measured by analyzing the bed effluent for arsenic-76. In all cases more than 98.5% of the arsenic content of the influent was retained by the bed.

To determine if loss of radiophosphorus was occurring on the copper sulfide, a phosphorus-32 spiked solution was passed through a 5 sq. cm. × 2 cm. bed. The activity of an aliquot of the effluent was measured with an end-window Geiger-Müller counter and was found to contain the same concentration of radiophosphorus as an equal portion of the influent solution. In addition, after washing with water, an aliquot of the bed was dried and the contained activity measured. This measurement showed that less than 0.1% of the radiophosphorus content of the influent was present on the total bed.

The use of diethyl ether as a diluent for 1-butanol was initiated to expedite sample preparation during the evaporation of the solvent. No study was made to determine the optimum concentration of 1-butanol in diethyl ether. The concentration reported was satisfactory for the intended purpose.

Exclusion of silicon-31 by extraction from 1.3*N* sulfuric acid was tested by measuring the decay of the final product. More than 99% of radiosilicon was removed from samples of reactor cooling water.

PROCEDURE

A 400-ml. sample of reactor effluent water was made 0.05*N* in hydrochloric acid and heated to boiling, and 1 ml. of 20% sodium thiosulfate solution was added. After standing for 5 minutes the solution was passed at a rate of 20 to 30 ml. per minute through a 5 sq. cm. × 2 cm. bed of cupric sulfide supported on a mat of asbestos. The first 100 ml. of column effluent was discarded. Two 100-ml. aliquots of the remainder were then analyzed. After

addition of 20 ml. of 10*N* sulfuric acid, 30 ml. of 10% ammonium molybdate reagent, and 0.25 mg. of phosphate carrier which served as an indicator, the solution was extracted by shaking with 35 ml. of 10% 1-butanol in diethyl ether. The organic phase was then separated and washed by shaking with 15 ml. of 1.3*N* sulfuric acid. After final separation, the organic phase was evaporated on a standard 1-inch stainless steel counting dish and the activity measured with an end-window Geiger-Müller counter.

RESULTS AND DISCUSSION

The reproducibility of this method was tested by analyzing a series of aliquots of a standardized phosphorus-32 solution. The results of two experiments, presented in Table I, show an average radiochemical recovery of 86.6% with a standard deviation of 5.2%. Analysis of these data, coupled with the fact that negligible phosphorus-32 sorption was found on the cupric sulfide bed, provides $E_a^0 = 6.5$, the distribution coefficient of molybdophosphoric acid in the system used. This distribution has proved constant for this procedure and was suitable for the intended purpose.

Table I. Reproducibility of Phosphorus Recovery from Spiked Samples

Run 1		Run 2	
Sample	Counts/min.	Sample	Counts/min.
1	1600	1	1631
2	1497	2	1599
3	1509	3	1460
4	1539	4	1567
5	1491	5	1525
6	1263	6	1471
7	1341	7	1595
8	1501	8	1377
9	1518	9	1506
10	1428	10	1492
11	1395	11	1585
12	1482	12	1471
13	1419	13	1459
14	1423	14	1555
15	1400	15	1568
16	1352		
Av.	1447		1524
	5.9%		4.5%
Theoretical	1689		1742
Average radiochemical yield	85.7%		87.5%
Over-all average yield	86.6%		
Standard deviation	5.2%		

A series of reactor effluent water samples was analyzed in duplicate for radiophosphorus to test the reproducibility of the procedure (Table II). Relative values for the radiophosphorus concentration are given, because the actual concentrations are classified information. The radioactive decay of these samples was followed for 70 days and in all cases exhibited a single component with a half life of 14.3 days, although the phosphorus-32 accounted for less than 1% of the initial sample activity. The radiochemical purity of these samples was evidenced by the absence of materials of a half life other than this value when the decay was followed for five half lives of phosphorus-32.

The sensitivity of this procedure is dependent on the operational characteristics of the counting equipment employed. In the case of the Geiger-Müller counters used for this work, sensitivity of 1.6×10^{-7} microcurie per ml. is attainable for a 100-ml. sample.

CONCLUSIONS

The procedure described for the separation and determination of phosphorus-32 from a variety of radionuclides afforded a final product of high radiochemical purity. Although only 86.6% of the radiophosphorus was recovered, the reproducibility was found to be satisfactory.

Table II. Determination of Phosphorus-32 in Reactor Effluent Water

Sample No.	Relative Activity	Av. Activity	% Deviation
1A	35.3		
1B	35.7	35.5	0.9
2A	38.7		
2B	38.4	38.6	0.8
3A	41.7		
3B	44.7	43.2	6.3
4A	41.1		
4B	41.4	41.3	0.8
5A	51.2		
5B	48.8	50.0	4.3
6A	49.1		
6B	50.4	49.8	2.0
7A	47.5		
7B	47.2	47.4	0.7
8A	38.7		
8B	43.9	40.3	11.2
9A	27.0		
9B	27.0	27.0	0.0
10A	19.7		
10B	22.7	21.2	12.8
11A	44.2		
11B	45.9	45.1	3.4
12A	38.0		
12B	41.1	39.5	6.8
13A	51.7		
13B	51.7	51.7	0.0
Average relative error	3.8%		

Efficient decontamination from all radioisotopes in reactor effluent was afforded following specific removal of arsenic-76 by sorption on a bed of cupric sulfide and exclusion of silicon-31 by utilizing its nonextractability from an acidic solution.

Application of this procedure for the determination of phosphorus-32 in many other types of samples is suggested, as the method is rapid and specific for phosphorus.

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RECEIVED for review February 20, 1956. Accepted August 1, 1956. Hanford Atomic Products Operation is operated by the General Electric Co. for the U. S. Atomic Energy Commission under Contract No. W-31-109-Eng-52.

Application of Lithium Aluminum Hydride to the Determination of Hydroxyl Groups

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Prior to the introduction of lithium aluminum hydride as a reagent for active hydrogen, no satisfactory method existed for the determination of hydroxyl in the presence of α-epoxide groups. Because accurate functional analysis of epoxy compounds (as exemplified by epoxy resins) is frequently necessary, the application of lithium aluminum hydride for this purpose was investigated. An improved method was developed and has been found valuable for the rapid and accurate determination of hydroxyl in epoxy compounds. The method is also useful in analysis of other materials, where the more conventional hydroxyl methods are limited.

THE reactions of lithium aluminum hydride with organic compounds and the methods used for their measurement have been discussed in a recent review article (*6*). The authors' experience with the reagent has demonstrated its value for the measurement of active hydrogen, in particular the determination of alcoholic and phenolic hydroxyl groups. The reaction is uniquely applicable to the determination of hydroxyl in the presence of epoxy groups as in Epon resins (Shell Development Co. trade-mark), where other hydroxyl methods fail. The technique is also useful for the analysis of hindered phenols which do not react under the conditions of the classical acetylation methods.

Lithium aluminum hydride is usually employed for active hydrogen determinations in procedures involving measurement of volume (*17*) or pressure (*9, 10*) of hydrogen evolved in the reaction. Higuchi (*6, 7*) describes a titrimetric procedure in which excess reagent is titrated with a standard solution of alcohol in xylene. Because the latter procedure measures consumption of reagent, the results are affected by all reactions of lithium aluminum hydride, many of which do not involve active hydrogen.

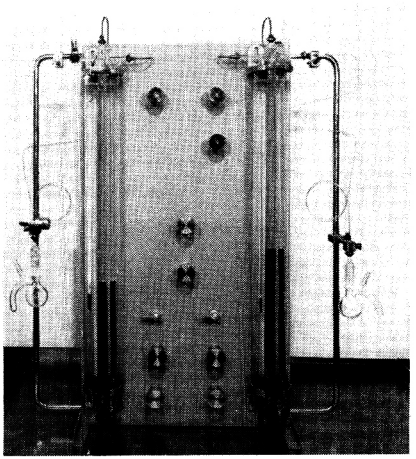
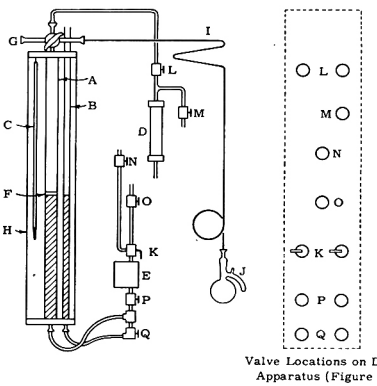


Figure 1. Dual lithium aluminum hydride apparatus



Valve Locations on Dual Apparatus (Figure 1)

Figure 2. Schematic diagram of apparatus

A.	100-ml. buret	*J.*	Reactor (see Figure 3)
B.	Leveling tube	*K.*	Three-way valve control for mercury lift steel needle valves
C.	Thermometer	*L.*	Nitrogen flushing
D.	Nitrogen dryer containing Dehydrite, to nitrogen source	*M.*	Control to tetrahydrofuran dispenser (see Figure 5)
E.	Mercury reservoir, 250 ml.	*N.*	Air supply
F.	Tetrahydrofuran layer	*O.*	Vacuum supply
G.	Vent port	*P.*	Buret
H.	Water-filled buret jacket	*Q.*	Leveling tube
I.	Stainless steel Hypotubing, 14-gage		

The equipment employed in this laboratory is a modification of the volume-measuring apparatus originally described by Zerewitinoff (*17*), a design which still appears good because of its simplicity. The major improvement made in this application lies in the substitution of stainless steel hypodermic tubing for glass and rubber tubing, resulting in increased flexibility and a reduction in dead space.

APPARATUS

A photograph and a schematic diagram of the apparatus are shown in Figures 1 and 2. The dual unit is designed from the standpoint of maximum convenience and efficiency.

Each unit consists of a 100-ml. water-jacketed gas buret graduated in 0.2-ml. divisions and equipped with a four-way L-bore stopcock, a leveling tube located in a position adjacent to the gas buret, and a mercury lift connected at the top by means of a three-way cock to vacuum and air services and at the bottom to the buret and leveling tube by means of steel needle valves and plastic tubing. The buret and mercury lift have been described in detail (*2*). The 14-gage stainless steel Hypotubing is looped as shown in Figure 1, and 5/12 steel spherical joints are soldered to its ends. The steel spherical joints are sealed to the glass joints of the four-way stopcock and of the reactor adapter (Figure 3) by sealing wax.

The reaction flask has a capacity of approximately 50 ml., with a side arm of approximately 10-ml. capacity, as illustrated in Figure 3. A standard-taper joint is provided on top of the side arm and the flask is attached to the system by means of an adapter.

The 250-ml. flask for preparation of reagent has a stoppered joint on top and a standard-taper joint and a stopcock near the top. The reagent storage flask is similar, but sealed at the top. A connecting piece is provided for filtration of the reagent from one vessel to the other. These parts, which are similar to those described by Zaugg and Lauer (*16*), are shown in Figure 4. The openings in the 19/38 standard-taper joints are closed by insert-

ing 12-mm. sleeve-type rubber serum stopples. A 5-ml. hypodermic syringe fitted with a 22-gage stainless steel needle is used for transferring reagent from storage vessel to reaction flask.

The storage and dispensing apparatus for tetrahydrofuran consists of a 1-liter borosilicate glass bottle having a 29/42 standard-taper joint into which two pieces of glass tubing are sealed, as shown in Figure 5. One piece of tubing, 12 mm. in inside diameter, has a standard-taper joint sealed at the top, into which a pipet is inserted; the pipet is provided with a stopcock and a small drying tube. The other piece of tubing, 6 mm. in inside diameter, is connected to a drying tube, small glass T-tube, and the source of nitrogen. With nitrogen flowing, the solvent is forced into the pipet by placing a finger over the open end of the T-tube.

REAGENTS

Tetrahydrofuran was chosen as a solvent because of its excellent solvent properties for many resinous materials as well as for lithium aluminum hydride.

With unknown samples having more than the expected quantity of reducible functional groups, it is possible to consume all of the reagent without obtaining quantitative reaction of active hydrogen. For this reason the reagent solution is made up to contain a trace of 4-phenylazodiphenylamine as an indicator to verify the presence of excess reagent. Higuchi (*6*) recommended the use of this compound as an indicator for titration of lithium aluminum hydride.

The Lithium Aluminum Hydride Reagent is an approximately 2.5*M* solution in tetrahydrofuran. Connect the flasks as shown in Figure 3 with a glass filter plug inserted in the connecting piece and flush completely with nitrogen. Add approximately 11 grams of lithium aluminum hydride, 100 ml. of purified tetrahydrofuran, and 0.15 ml. of 1% 4-phenylazodiphenylamine solution to the reagent preparation flask. Stopper the flask and, with the stopcocks open, swirl vigorously. After most of the lithium aluminum hydride has dissolved, close the stopcock and allow the solution to stand overnight. Filter the reagent into the storage flask, maintaining slight nitrogen pressure to facilitate the transfer. Disassemble the apparatus and close the storage flask by inserting a serum stopple into the standard-taper joint.

CAUTION. Wear a face mask during this operation and keep the reagent off the skin. Detailed safety precautions should be obtained from the manufacturer, Metal Hydrides, Inc., Beverly, Mass.

Tetrahydrofuran. Prior to purification, tetrahydrofuran should be tested for peroxide content by an iodometric method (*15*) and rejected for use in this technique if the peroxide oxygen is greater than 10 meq. per liter. Commercially available material in previously unopened containers is generally satisfactory in this respect.

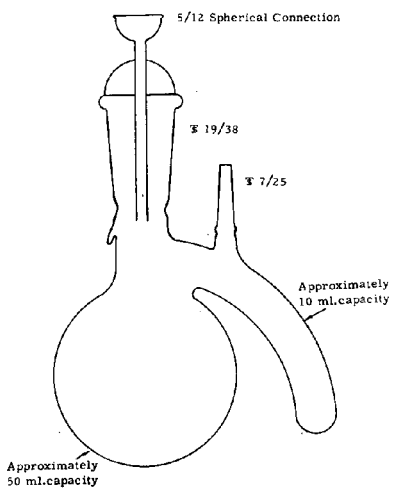


Figure 3. Reaction flask assembly

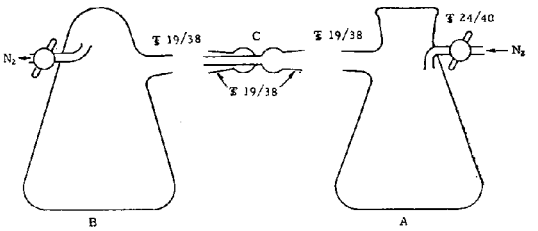


Figure 4. Diagram of apparatus for preparation and storage of lithium aluminum hydride reagent

A. 250-ml. flask for preparation of reagent
B. 250-ml. flask for storage of reagent
C. Connecting piece for filtration of reagent from *A* to *B*

Table I. Maximum Sample Sizes

Estimated Hydroxyl Content, Eq./100 G.	Sample Size, Grams
0.1	2.0
0.5	0.4
1.0	0.2

To purify, place 1 liter of tetrahydrofuran in a dry 2-liter round-bottomed flask fitted with a standard-taper joint. Displace the air from the flask with dry nitrogen and maintain a slow stream of nitrogen over the solvent. Add lithium aluminum hydride in small increments, with swirling. Continue the addition until an excess is present, as indicated by the absence of further gas evolution (10 to 20 grams will usually suffice). Connect the flask to a distilling head and condenser, and distill the tetrahydrofuran, using a mantle-type heater. Discard the first 50 ml. of distillate, and collect approximately 800 ml. in a 1-liter borosilicate glass dispensing bottle (Figure 4) from which air is excluded by a slow stream of dry nitrogen. Discontinue the distillation when approximately 100 ml. of liquid remains in the flask. Protect the distillate from moisture by a drying tube immediately upon completion of the distillation.

4-Phenylazodiphenylamine. 1% solution in benzene.

GENERAL OPERATING TECHNIQUES

Operation of Mercury Lift. The following operations are illustrated by Figure 2.

A. To raise the mercury level in the buret, close valve *Q*, turn three-way valve *K* to "air" position, and open valve *P*.

B. To lower the mercury level in the buret, close valve *Q*, turn three-way valve *K* to "vacuum" position, and open valve *P*.

C. To raise or lower the mercury levels in both buret and leveling tube, carry out operation A or B with valve *Q* open.

D. To level the mercury columns, open valve *Q*, turn three-way valve *K* to "vacuum," and open valve *P*. When the mercury level in the leveling tube is below that in the buret, turn three-way valve *K* to "air," and close valve *P* to a low flow. Close valve *P* completely when the columns have risen to the same level.

Buret Maintenance. Place a layer of purified tetrahydrofuran in the buret to ensure saturation of the contained gas. Replace the tetrahydrofuran weekly with fresh material. Do not allow the liquid to come in contact with the buret stopcock, as it will dissolve the lubricant.

Testing for Leaks. Prior to performing any analyses, test the apparatus for leaks. Attach a reaction flask to the apparatus. Raise the mercury in the buret to a level near the top, venting the gas to the atmosphere. Turn the stopcock to connect the buret with the reaction flask. Lower the mercury level in the buret to approximately the 20-ml. mark and note the volume. Wait 3 to 4 minutes and again note the volume. If a change is observed, carefully relubricate the buret stopcock and glass joints and test again. Read the top level of the tetrahydrofuran in the buret when measuring the volume.

Glassware Preparation. Dry all glassware in an oven at 110° C. for 30 minutes and store in a desiccator.

Sample Size. Use an amount of sample calculated to yield 60 to 80 ml. of hydrogen; maximum sample sizes for typical anhydrous samples are given in Table I. Limit the size of sample to 0.5 gram, unless the behavior of the material with respect to side reactions is known.

Table II. Reaction of Alcohols and Phenols

Compound	Hydroxyl Value, Eq./100 G. Theoretical	By independent method, av.	By $LiAlH_4$
Cetyl alcohol	0.413	0.410[a]	0.411, 0.412, 0.413
4-Methyl-4-pentene-2-ol	0.999	0.984[b]	0.982
tert-Amyl alcohol	1.135	1.124[c]	1.11
Ethylene glycol	3.223	3.194[a]	3.188
Phenol	1.064	....	1.062, 1.064
2,6-Dibutyl-4-methylphenol	0.454	0.452, 0.449[d]	0.456, 0.454
2,4,6-Tributylphenol	0.381	0.389[d]	0.376, 0.373
2,2-Bis(*p*-hydroxyphenyl)propane	0.874	0.863[e]	0.861
Tris(*p*-Hydroxyphenyl)-methane	1.026	1.1[d]	1.020

[a] Acetic anhydride method (*14*).
[b] Acetyl chloride method (*12*).
[c] Boron trifluoride–Fischer reagent method (*3*).
[d] Ethylenediamine titration (*5*).
[e] Calculated from calorimetric purity measurement (*13*).

PROCEDURES

General Procedure. Into a dry reaction flask previously flushed with dry nitrogen, weigh, to the nearest milligram, the appropriate amount of sample as given in Table I. Add 5.0 ml. of tetrahydrofuran, stopper the flask, and swirl gently until the material has dissolved. By means of a hypodermic syringe, introduce 5 ml. of lithium aluminum hydride reagent into the side arm of the flask through the standard-taper joint on the top of the side arm and cap the standard-taper joint.

Flush the gas-measuring apparatus with nitrogen for approximately 5 minutes, uncap the joints on the reaction flask, and immediately attach the flask to the system. Allow nitrogen to pass through for approximately 1 minute at a slow rate, shut off the nitrogen, and cap the small joint on the side arm. Turn the buret stopcock to connect with the reaction flask, immerse the flask completely in an ice slurry kept in a Dewar flask, and balance the levels of mercury. Allow several minutes for the system to equilibrate; equilibrium is established when the levels of mercury in the buret and leveling tube remain constant for approximately 5 minutes. Turn the buret stopcock to connect with the atmosphere, and force the liquid level in the buret up to the zero mark, expelling the nitrogen from the buret into the atmosphere.

Turn the buret stopcock to connect with the reaction flask, set the three-way valve of the mercury lift on "Vacuum," and open the lower needle valve. Raise the reaction flask out of the ice bath, open the upper needle valve slightly, and tip the flask so that a portion of the reagent in the side arm flows into the sample solution. Swirl the flask and continue adding reagent in small increments, with swirling, until all has been used. Maintain the system at as near atmospheric pressure as possible at all times.

When all gas evolution has ceased, replace the reaction flask in the ice bath, close the upper needle valve, and equilibrate for about 10 minutes; establish atmospheric pressure in the buret. When the mercury levels in the buret and leveling tube remain constant for 5 minutes, read and record the buret volume, jacket temperature, and barometric pressure. The color of the reaction mixture should be red. If the solution is yellow, indicating depletion of reagent, repeat determination with a smaller sample.

Resin Procedure. Into a dry reaction flask previously flushed with dry nitrogen, weigh, to the nearest milligram, the appropriate amount of sample as given in Table I. Introduce a $^1/_2$-inch steel ball in the flask, add 5.0 ml. of tetrahydrofuran, swirl gently to dissolve the sample, and add 5.0 ml. of tetrahydrofuran. By means of a hypodermic syringe, introduce 5 ml. of lithium aluminum hydride reagent into the side arm of the flask, cap the standard-taper joint, and proceed as directed in the general procedure.

After addition of the reagent, mix the solution thoroughly, swirling the flask in such a manner that the steel ball will aid in dispersing any precipitate which is formed. Immerse the flask in the ice bath, allow several minutes for equilibration, establish atmospheric pressure in the buret, and record the gas volume and jacket temperature. Allow the mixture to react until a constant volume is obtained; in general, resins require reaction times of from 0.5 to 2.5 hours. Mix the contents of the flask thoroughly several times during the reaction and take gas volume readings every 15 to 30 minutes. When analyzing materials which require extended reaction times it is advisable to leave the three-way valve in the "vacuum" position between volume readings.

Additional Tests. BLANK. Make a blank determination exactly as described above, omitting the sample, and using the same volume of tetrahydrofuran as for the sample determination. The blank volume for 5 ml. of solvent is normally 1 to 5 ml. of gas.

WATER. Determine the water content of the sample by titration with Fischer reagent. The water determination should be made preferably on the same day the hydroxyl value is determined.

ACIDITY. Determine the acidity of the material by titration with standard base to the phenolphthalein end point.

CALCULATION

Calculate the hydroxyl value of the sample by means of the following equation:

$$\text{Hydroxyl value, eq./100 g.} = \frac{(S - B)\,1.604\,(P - Q)}{(T + 273)\,W\,(1000)} - \left(\frac{C}{9} + D\right)$$

where

S = volume of gas evolved by the sample, milliliters
B = volume of gas evolved by the blank, milliliters
P = atmospheric pressure, millimeters
Q = vapor pressure of tetrahydrofuran, millimeters, calculated from the expression, $Q = 6.8T - 1$
T = jacket temperature, degrees centigrade
W = weight of sample, grams
C = water content, per cent by weight of the sample
D = acid content of the sample, equivalents per 100 grams
1.604 = reciprocal of the gas constant
9 = equivalent weight of water in this determination

RESULTS AND DISCUSSION

Alcoholic and phenolic hydroxyl groups react to produce 1 mole of hydrogen per equivalent of hydroxyl. These reactions are rapid, in most cases reaching completion within 10 minutes at 0° C. as shown in Table II. Excellent accuracy is observed in the analysis of primary, secondary, and tertiary alcohols. Values obtained for phenol and highly substituted phenols indicate stoichiometric reaction. The method has been found especially useful for analysis of ortho-substituted phenols, which react only partially or not at all with esterification reagents such as acetyl chloride (*12*) or acetic anhydride (*14*).

In the authors' experience, water reacts to a variable degree under the conditions of the method, giving 1.5 to 2.0 moles of hydrogen per mole. The extent of reaction is influenced by the presence of other reactants; water by itself in an inert solvent yields 1.5 moles of hydrogen per mole, in agreement with Baker and MacNevin's observations (*1*); small amounts of water in the presence of other hydroxyl compounds produce 1.9 to 2.0 moles of hydrogen, as observed by Hochstein (*8*). When the method is applied to samples low in water content, correction for water is made assuming equivalent weight of 9 (2 equivalents per mole).

Table III. Reaction of Other Common Organic Functional Groups

Compound	Moles/100 G., Calcd.	Active Hydrogen, Eq./100 G. ($LiAlH_4$)
Acetone	1.724	<0.01
Acetophenone	0.833	0.008
Hexaldehyde	1.00	0.002, 0.001
Benzaldehyde	0.943	0.003
Glycidyl phenyl ether	0.666	<0.001
Glycidol	1.349	1.357
Epichlorohydrin	1.307	0.002
Benzoic acid	0.819	0.827, 0.828, 0.828, 0.832
Oleic acid	0.354	0.352, 0.353, 0.354
Ethyl acetoacetate	0.769	0.47, 0.51
Diethyl malonate	0.624	0.51, 0.58

Table IV. Reaction of α-Epoxide Group under Conditions of Hydroxyl Methods Involving Esterification

Compound	Apparent Reaction of Epoxide Group, % Acetyl chloride[a]	Acetic anhydride[b]	BF_3 Fischer[c]
Glycidyl phenyl ether	4, 5	26	65, 79
Glycidol	16, 42	98, 44	10, 36
Epichlorohydrin	9, 9	32	98

[a] Smith and Bryant method (*12*).
[b] Verley and Bölsing method (*14*).
[c] Bryant, Mitchell, and Smith method (*3*).

Simple aldehydes and ketones are reduced to the corresponding primary and secondary alcohols, without the evolution of hydrogen. Epoxides are reduced in a similar manner. As shown in Table III no active hydrogen was observed in the reaction of several simple carbonyl compounds and epoxides. Glycidol, an epoxy alcohol, produced hydrogen equivalent to the hydroxyl content. In contrast, the typical interference encountered in the application of three esterification procedures to epoxide samples is shown in Table IV. Carboxylic acids react to produce 1 mole of hydrogen per mole. In addition, carboxylic acids are reduced to alcohols without the production of hydrogen. Values found for benzoic and oleic acid, shown in Table III, demonstrate the stoichiometric production of hydrogen from these compounds. Hydrogen produced on reaction of lithium aluminum hydride with the tautomeric compounds ethyl acetoacetate and diethyl malonate indicates 60 to 80% reaction as the enol form. It has previously been reported (*18*) that these compounds react with Grignard reagent as though completely enolized. Apparently the keto form is reduced by lithium aluminum hydroxide sufficiently rapidly to prevent complete enolization.

Application to Epoxy Resins. Prior to the introduction of the lithium aluminum hydride technique, no satisfactory method existed for the determination of hydroxyl in the presence of epoxides—for example, in Epon (*11*) resins—owing to a considerable and variable reactivity of epoxide groups under the conditions of the methods existing at that time. Epon resins, which are polymers prepared by condensation of epichlorohydrin with polyhydric phenols or alcohols, contain unreacted epoxy groups and hydroxyl groups. These resins and modifications thereof are produced in various degrees of polymerization and chemical composition. The demonstrated noninterference of epoxides in the lithium aluminum hydride method makes it uniquely applicable to this type of sample. As a result, the present most extensive use of the method is for the determination of hydroxyl in Epon resins. Results obtained in the analysis of several typical Epons and other epoxy resins are shown in Table V. The precision obtained by the method is demonstrated by the agreement among multiple values obtained for several Epon resins and for a polymer of allyl glycidyl ether. Similar precision is obtained for many experimental resins of Epon type.

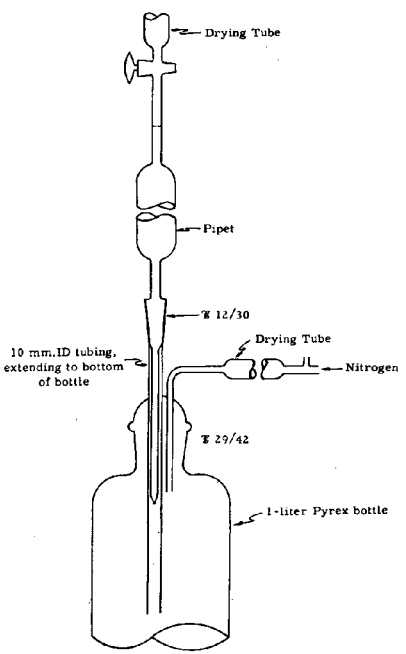


Figure 5. Storage and dispensing system for tetrahydrofuran

Table V. Determination of Hydroxyl in Typical Resins by Lithium Aluminum Hydride Method

Material	Hydroxyl Content, Eq./100 G.
Epons	
828	0.091, 0.094
1001	0.262, 0.259, 0.264
1004	0.305, 0.315, 0.305, 0,310
1007, lot 1	0.360, 0.353
1007, lot 2	0.347, 0.342
Poly (allyl glycidyl ether)	0.108, 0.108

Table VI. Analysis of Cured Epon 828 Powder Fractions

(Resin cured with triethylamine, ground in a small hammer mill, and screened to obtain fractions shown)

Mesh Size No.	Hydroxyl Content, Eq./100 G.	Epoxide Content, Eq./100 G.
>60	0.054	0.026
60–100	0.056	0.036
100–200	0.061	0.047
200–325	0.080	0.056
<325	0.113	0.095

Dannenberg and Harp (*4*) described methods for determination of residual epoxy groups in insoluble cured Epon resins. Their technique for preparation of samples involved grinding to particle sizes of a few microns. Good agreement was found between infrared values and values obtained by reaction of the epoxy groups with hydrochloric acid in a dioxane medium, showing that diffusion of the reagent into the swelled solid was complete under the conditions employed. In the course of this work several fractions obtained by screening a powdered cured Epon were analyzed for hydroxyl content. The results are presented along with epoxide values in Table VI. Although the samples remained undissolved, appreciable reaction took place, even with the coarser samples. The hydroxyl values increase with decreasing particle size and it appears reasonable to assume that the value for the finest fraction comes close to the true hydroxyl content of the material.

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RECEIVED for review May 9, 1956. Accepted July 30, 1956.

Determination of Neomycins B and C in Neomycin Sulfate

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This procedure for the determination of neomycins B and C in neomycin sulfate involves measurement of the optical rotation and determination of furfural produced from the pentose portions of the molecules by acid degradation. This procedure is compared with two other methods: measurement of change of optical rotation with temperature and titration of free base combined with optical rotation. The furfural procedure is preferred. It has an accuracy on pure mixtures within about 1% on total neomycin, 3% on neomycin B, and up to 10% on neomycin C (when small percentages of C are involved). The errors on finished neomycin are about 2% on the total and about 5% on neomycin B.

THE development of a suitable chemical assay has been hampered by the lack of complete knowledge of the structure of the neomycins and by the presence of functionally similar impurities. Neomycins B and C have been shown to be hexaamino compounds consisting of neamine, a pentose, and a diaminohexose (*1*, *2*, *4*, *6*) and possessing the empirical formula $C_{23}H_{48}N_6O_{13}$. Structural differences appear to reside in the diaminohexose.

Several procedures for the determination of total neomycin have been described. The colorimetric ninhydrin method of O'Keefe and Russo-Alesi (*7*) measures only total amino groups. Hamre and associates (*5*) determined total neomycins by an anthrone reaction, indicating equivalent responses by B and C. Dutcher, Hosansky, and Sherman (*3*) estimated the furfural produced by acid degradation of the neomycins and found that B produced about twice as much furfural as did C.

Two methods for the analysis of neomycin B-C mixtures have been published: the microbiological procedure of Sokolski and Carpenter (*9*) and the semiquantitative papergram method of Pan and Dutcher (*8*) for *N*-acetyl derivatives on neomycins B and C.

Because the specific rotations of neomycins B and C are different, a combination of a determination of optical rotation with a second measurement in which the two compounds respond in a different ratio should permit an analysis of B-C mixtures.

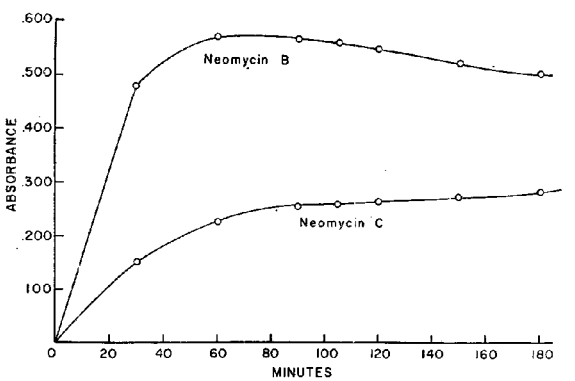

Figure 1. Development of furfural absorbance *vs.* time

Among the reactions which might serve to distinguish between the two molecules, the formation of furfural or substituted furfural (*2*, *3*) in sulfuric acid seemed the most promising. The neutral equivalent also seemed to be a reasonable method on which to base a set of simultaneous assays. Later it was found that the difference in the temperature coefficient of optical rotation could be used as an assay procedure. Three procedures based on the above observations are presented.

EQUIPMENT

A standard model Beckman DU spectrophotometer equipped with hydrogen lamp and 1-cm. quartz cells was used for absorbance measurements. The optical rotations were determined on a Gaertner polarimeter using a 2-dm. tube, except when the temperature variation was measured in a 4-dm. Landolf-Clerget tube through which thermostated water was circulated. Titrations were carried out with a Beckman Model G pH meter with glass electrode, using semimicroequipment under a carbon dioxide–free helium blanket.

REAGENTS

Neomycins B and C Sulfates. The materials used as standards are the same as described by Ford and coworkers (*4*, *10*) and are believed to be the purest materials available. The specific optical rotation ($[\alpha]_D^{25}$ in 0.1*N* sulfuric acid) and the equivalent weight of the free neomycin bases are defined as follows: neomycin B, +83.2°, 102.95; neomycin C, +120.8°, 102.95.

All other chemicals are reagent grade.

METHOD I. OPTICAL ROTATION–FURFURAL RESPONSE

Optical Rotation. The optical rotations are determined at ca. 25° C. on solutions that contain about 5 grams of neomycin sulfate in 25 ml. of 0.1*N* sulfuric acid. All rotations are taken at the Na*D* line. The rotations are corrected for temperature variation by the following formulas:

Neomycin B

$$\alpha_D^t = \alpha_D^{25}\,[1 - 0.00171\,(t - 25)] \quad 0° < t < 80° \text{ C.} \qquad (1)$$

Neomycin C

$$\alpha_D^t = \alpha_D^{25} \qquad 25° < t < 80° \text{ C.} \qquad (2)$$

$$\alpha_D^t = \alpha_D^{25}\,[1 + 0.000112\,(t - 25)^2] \quad 0° < t < 25° \text{ C.} \qquad (3)$$

Furfural Reaction. The furfural reaction is run on a solution containing not more than 1 gram of base per liter. This solution is obtained by quantitative dilution with water of the solution used for optical rotation. Three milliliters of the diluted solution are placed in a glass-stoppered test tube and cooled in an ice bath for several minutes. To this tube, still immersed in ice water, is added 2 ml. of concentrated (specific gravity 1.84) sulfuric acid. The solutions are mixed and cooled again in an ice bath. The stoppered tube is then placed in a boiling water bath—with the water level above the liquid level in the tube—for 100 ± 2 minutes and then quenched in ice water. The absorbance of a 1 to 10 dilution of this solution with water is then measured at the furfural peak (~277) mμ in a 1-cm. quartz cell against a 4% sulfuric acid blank which has been prepared from the concentrated acid in a manner identical to the sample.

The absorptivities of acid-degraded neomycins B and C are determined from known solutions of the standards using the same procedure. The calculations are made in terms of the diluted neomycin solution.

Calculations. Calculations based on the diluted neomycin solution are as follows:

$$B = \frac{c\alpha - \alpha_C^{25} A}{c\alpha_B^{25} - b\alpha_C^{25}} \quad (4)$$

$$C = \frac{\alpha_B^{25} A - b\alpha}{c\alpha_B^{25} - b\alpha_C^{25}} \quad (5)$$

where

B and C = concentrations of neomycins B and C free bases, grams per liter
b and c = absorptivities (Beer's law constants) of acid-degraded pure neomycins B and C free bases at ~277 mμ, liters per gram-centimeter
α_B^{25} and α_C^{25} = optical rotations of pure neomycins B and C free bases at 25° C., ° liter per gram-dm. (α_B^{25} = 0.0832°; α_B^{25} = 0.1208°)
A = observed absorbance at 277-mμ furfural peak
α = optical rotation for diluted neomycin solution calculated from observed rotation of concentrated solution

METHOD II. VARIATION OF OPTICAL ROTATION WITH TEMPERATURE

Optical rotation is measured on the solution described in Method I, using a 4-dm. jacketed tube maintained at ca. 75° and 25° C.

The concentration of neomycins is calculated from the following expressions:

$$B = \frac{\alpha^{t_2} - \alpha^{t_1}}{0.00171\,[\alpha_B]^{25}\,(t_1 - t_2)} \quad (6)$$

$$C = \frac{\alpha^{t_1} - [\alpha_B]^{t_1} B}{[\alpha_C]^{25}} \quad (7)$$

METHOD III. OPTICAL ROTATION–NEUTRAL EQUIVALENT

Optical rotation at 25° C. is measured as in Method I. In addition, a 5-ml. aliquot of the sample solution is mixed with an excess of saturated barium hydroxide solution and back-titrated with 0.2N sulfuric acid. Inflection points are determined by the method of equal increments and consist of a sharp end point at approximately pH 4.0 and a less sharp inflection at about pH 10.5.

Total neomycin is calculated from the normality, while the % B is read from a plot of α_D^{25}/N *vs.* % B, which was shown to be linear.

Table I. Assay of Neomycin B-C Mixtures by Method I

No.	Known Concn., Grams Base/Liter B	C	Total	Absorbance × 10^3 ± St. Dev. (n)	Calcd. α_D after Dilution	Found Concn., Grams Base/Liter B	C	Total
1[a]	1.000	...	...	543 ± 3.5 (3)	0.0832[b]	...	...	...
2[a]	...	1.000	...	269 ± 4.0 (3)	0.1208	...	...	...
3	0.775	0.190	0.965	484 ± 2.0 (3)	0.0879	0.806	0.172	0.978
4	0.613	0.374	0.987	446 ± 1.0 (3)	0.0962	0.648	0.350	0.998
5	0.530	0.521	1.051	442 ± 2.7 (3)	0.1074	0.567	0.522	1.089
6[a]	1.000	...	...	542 ± 0.7 (7)	0.0832[c]	...	...	...
7[a]	...	1.000	...	267 ± 1.5 (7)	0.1208	...	...	...
8	0.900	0.100	1.000	519 ± 1.9 (5)	0.0870	0.913	0.091	1.004
9	0.800	0.200	1.000	495 ± 1.0 (5)	0.0907	0.822	0.185	1.007
10	0.700	0.300	1.000	463 ± 1.7 (5)	0.0945	0.710	0.293	1.003

[a] Reference samples.
[b] Optical rotations calculated from readings on concentrated solutions.
[c] Optical rotations calculated from known standards used to prepare above mixtures.

DISCUSSION

The results of the optical rotation–furfural response method applied to several known samples are summarized in Table I. The results of the application of all three methods, as well as a bioassay applied to a set of typical bulk neomycin preparations, are given in Table II. Figure 1 presents the data required to establish the optimum time for acid degradation. It was established from the data of Table I that the optical rotations of neomycins B and C are additive. The ultraviolet absorption of the furfural at the 277-mμ peak obeys Beer's law to at least 1 gram of base per liter in the undiluted, acid-degraded solution.

Table II. Assay of Typical Bulk Neomycin Sulfates[a]

(Gram of total neomycin bases per gram of sample and % neomycin B based on total neomycins)

Sample	Method I	Method II	Method III	Average	Bio-assay[b]
1	0.644 81.6	0.614 91.8	0.710 102.5	0.656	0.675
2	0.615 94.1	0.627 99.0	0.650 106	0.631	0.705
3	0.677 101	0.654 91.5	0.700 108	0.677	0.787
4[c]	0.785 93.9	0.772 89.9	0.781 92.0	0.779	0.768
5	0.691 96.3	0.665 88.6	0.683 97.0	0.680	0.714

[a] Neomycin sulfate used contains about 64% neomycin free base calculated from degree of neutralization.
[b] *K. pneumoniae* turbidimetric assay described in (*10*).
[c] Neomycin hydrochloride.

The results on the known samples lie within the propagated experimental error and the scatter is reduced by use of a 100-minute degradation time. The errors on total neomycin of about 1%, on neomycin B of 3%, and on neomycin C of about 10% are satisfactory, considering the effect of a simultaneous assay on the propagation of errors.

The results of the three methods as applied to typical finished neomycin preparations are in good agreement for total neomycin. The deviation from the average is the least for the optical rotation–furfural response method and is random in character. The deviations of the other two methods are not random. The agreement with the biological assay is not so good as the agreement between the physical-chemical methods, but the difference is not unduly large.

The values for the percentage of neomycin B show a considerable scatter among the three methods. It is difficult to determine the influence of the interfering impurities present in typical finished neomycin powders. In most cases the impurities are unknown, although the method of preparation would lead one to expect the presence of optically active or inactive weak bases. Both of these would contribute to high values of total neomycin and probably to high percentages of B by the optical rotation–neutral equivalent method.

It is difficult to predict the effects of impurities upon the change of optical rotation with temperature. The presence of furfural-yielding impurities is less likely than the presence of optically active or inactive weak bases. The effect upon the optical rotation–furfural response method could be in either direction, although the effects tend to be self-compensating in the total neomycin value. On the basis of the present data the effects of impurities would appear to be less than about 2% on total neomycin and less than about 5% on the percentage of neomycin B.

The optical rotation–furfural response and optical rotation–neutral equivalent methods lend themselves to large numbers of routine samples, whereas the measurement of optical rotation at 75° C. is slow and awkward. The preferred method would appear to be the optical rotation–furfural response procedure.

ACKNOWLEDGMENT

The authors are indebted to the Department of Biological Control for the bioassays reported.

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RECEIVED for review April 25, 1956. Accepted July 20, 1956.

CRYSTALLOGRAPHIC DATA

139. 2-Amino-2-methyl-1,3-propanediol

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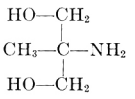

Structural formula for 2-amino-2-methyl-1,3-propanediol

2-AMINO-2-methyl-1,3-propanediol can be crystallized from acetone or from the lower alcohols. The crystals used in this study were obtained by allowing a warm isopropyl alcohol solution to cool. The resulting crystals are needles and rods elongated parallel to the *c* axis.

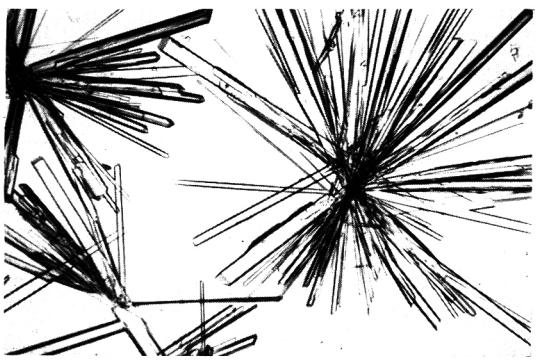

Figure 1. Typical crystals of 2-amino-2-methyl-1,3-propanediol recrystallized on microscope slide from isopropyl alcohol

The fusion behavior of this compound is like that of tris-(hydroxymethyl)aminomethane, which appeared in this column previously (*1*). When 2-amino-2-methyl-1,3-propanediol is heated, it loses birefringence sharply at 83–4° C., but retains its external form. The final melting point marked by loss of form is at 110–2° C.

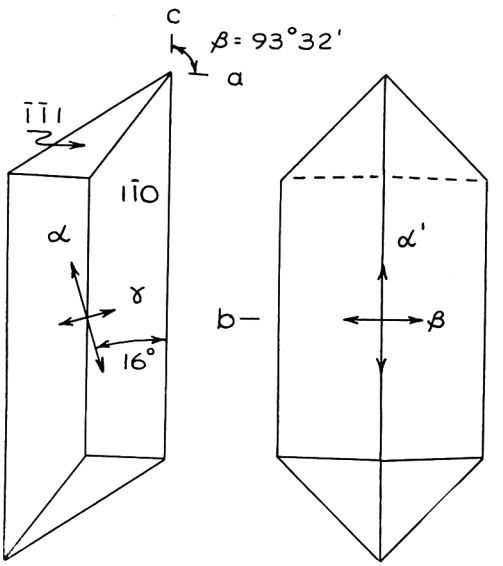

Figure 2. Orthographic projection of 2-amino-2-methyl-1,3-propanediol

The x-ray powder diffraction data were obtained using a camera 114.6 mm. in diameter and chromium radiation with vanadium filter. A wave length value of 2.2896 A. was used in the calculations.

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habits. Needles or rods elongated parallel to *c* and showing the prism {110} and positive hemibipyramid; upper {$\bar{1}11$} with ($\bar{1}11$) and lower {$11\bar{1}$} with ($1\bar{1}\bar{1}$).

Interfacial Angles (Polar). 110 ∧ $\bar{1}10$ = 103°58′ (calculated x-ray), 104°12′ (observed optical).

Beta Angle. 93°32′.

X-Ray Diffraction Data

Cell Dimensions. a_0 = 8.62 A., b_0 = 11.00 A., c_0 = 6.10 A.
Formula Weights per Cell. 4.
Formula Weight. 105.14.
Density. 1.211 grams per cc. (displacement), 1.211 grams per cc. (x-ray).

Optical Properties

Refractive Indices (5893 A., 25°C.), α = 1.516, β = 1.528, γ = 1.538.
Optic Axial Angle. $2V$ = (−) 84°24′ (calculated from α, β, and γ).
Optic Axial Plane. Perpendicular to 010.
Acute Bisectrix. α.
Extinction. $\alpha \wedge c$ = 16° in acute β.

X-Ray Powder Diffraction Data

d	I/I_1	$h\ k\ l$	d (Calcd.)
6.81	0.50	110	6.78
5.33	0.80	011	5.33
5.12	0.50	10$\bar{1}$	5.12
4.83	0.80	101	4.83
4.65	1.00	11$\bar{1}$, 120	4.64, 4.63
4.30	0.40	200	4.30
4.08	1.00	021	4.08
3.75	0.20	12$\bar{1}$	3.75
3.43	0.20	21$\bar{1}$	3.44
3.37	0.20	220	3.39
3.26	0.40	211	3.26
3.14	0.20	031	3.14
2.98	0.20	13$\bar{1}$	2.98
2.90	0.30	221	2.90
2.73	0.10	112	2.73
2.66	0.10	30$\bar{1}$	2.66
2.57	0.10b	23$\bar{1}$, 20$\bar{2}$	2.58, 2.56
2.46	0.20	311	2.47
2.41	0.10	20$\bar{2}$	2.42
2.39	0.10	14$\bar{1}$	2.43
2.35	0.20	212	2.36
2.23	0.05		
2.04	0.05		
1.990	0.20		
1.905	0.05		
1.850	0.05		
1.795	0.05		

Fusion Behavior. On heating, 2-amino-2-methyl-1,3-propanediol loses birefringence but not form at 83–4°. On continued heating the reduction to a melt takes place at 110–112° C. The melt does not crystallize on cooling.

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(1) Rose, H. A., Van Camp, A., Anal. Chem. 27, 1356 (1955).

140. Lead Azide, $Pb(N_3)_2$ (Form I)

Contributed by KIYO HATTORI and WALTER McCRONE[1],

Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

Form I (alpha) of lead azide is the polymorphic form stable at room temperature. It is prepared by mixing equal volumes of 1M lead nitrate and 2M sodium azide. Excellent crystals are then obtained by recrystallizing this crude precipitate from 15% (by weight) aqueous sodium acetate. The solution, saturated at about 50° C., is filtered and allowed to cool slowly without agitation over a period of several hours. Typical crystals prepared in this manner are shown in Figures 1 and 2.

The positions of the lead atoms in each of the polymorphic forms have been determined by Azaroff (*1*). Crystals of Form II transform readily to Form I in solution (Figure 3).

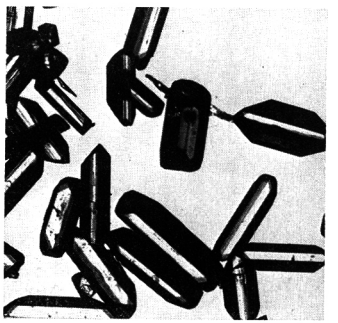

Figure 1. Typical crystals of lead azide I recrystallized from aqueous sodium acetate

Principal Lines

d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1
5.37	5	2.81	9	1.996	8	1.520	5
5.01	4	2.75	2	1.955	7	1.511	1
4.84	10	2.69	8	1.920	6	1.493	<1
4.64	4	2.63	<1	1.894	6	1.476	3
4.44	<1	2.58	6	1.868	3	1.458	6
4.27	6	2.55	7	1.833	7	1.428	4
				1.806[a]	6		
4.13	8	2.48	6	1.779	6	1.414	5
3.90	5	2.34	<1	1.726	5	1.385	7
3.78	6	2.28	7	1.691	5		
3.29	4	2.16[a]	3	1.657	5		
3.20	4	2.14[a]	4	1.627	5		
3.15	3	2.12	1	1.604	3		
3.08	9	2.09	7	1.584	6		
3.02	3	2.06	6	1.562	4		
2.95	6	2.03	6	1.538	4		

[a] Not well resolved.

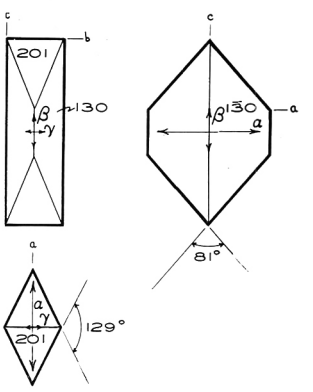

Figure 2. Orthographic projection of typical crystal of lead azide

[1] Present address, 500 East 33rd. St., Chicago 16, Ill.

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.
Form and Habit. Crystals from sodium acetate solution are usually elongated parallel to c with the prism {130} and macrodome {201}. Occasionally the prism {110} also appears.
Axial Ratio. $a:b:c = 0.700:1:0.408$.
Interfacial Angles (Polar). $201 \wedge 20\bar{1} = 81° 10'$. $130 \wedge \bar{1}30 = 50° 56'$. $110 \wedge 1\bar{1}0 = 69° 56'$.

X-RAY DIFFRACTION DATA

Space Group. $Pc2_1n$ or $Pcmn$ (*1*).
Cell Dimensions. $a = 11.41$ A.; $b = 16.31$ A.; $c = 6.66$ A. $a = 11.33$ A.; $b = 16.22$ A.; $c = 6.66$ A. (*1*).
Formula Weights per Cell. 12 (12.07 calculated from x-ray data).
Formula Weight. 291.3.
Density. 4.71 [displacement in benzene (*2*)]; 4.68 (x-ray).

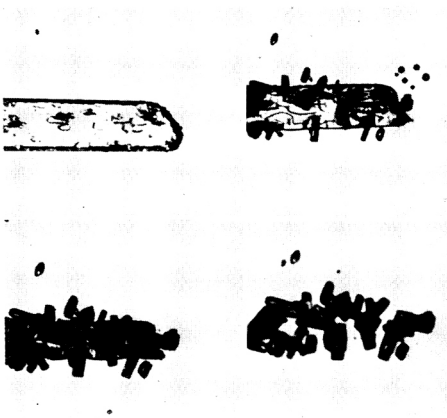

Figure 3. Four successive photomicrographs during solution phase transformation of lead azide II (*upper left*) to lead azide I (*lower right*)

OPTICAL PROPERTIES

Refractive Indices (5893 A.; 25° C.). $\alpha = 1.86 \pm 0.01$. $\beta = 2.24 \pm 0.01$. $\gamma = 2.64 \pm 0.01$.
Optic Axial Angles (5893 A.; 25° C.). $2V = 76.6°$ (calculated from α, β, and γ).
Dispersion. $v > r$.
Optic Axial Plane. 001.
Sign of Double Refraction. Positive, as calculated from refractive indices; negative, as observed conoscopically (see note below).
Acute Bisectrix. $\alpha = a$.
Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 2.22$; R (obsd.) = 35.1.

Note. Explanation of Anomalous Sign of Double Refraction. There are two conventions used to classify crystals as optically positive or optically negative. The usual convention is based on the refractive indices, according to which if α-β is greater than β-α the crystal is optically positive. On the other hand, the sign is often based on conoscopic observations, according to which Bx_a will be γ for negative crystals. These two criteria, however, lead to opposite signs for $Pb(N_3)_2I$. This situation arises only with compounds having very high birefringence and $2V$ close to 90°.

Lead Azide, $Pb(N_3)_2$ (Form II)

SUITABLE crystals of lead azide, Form II, are prepared by slow diffusion of lead and azide ions, using the apparatus shown in Figure 4. The pure water phase surrounding the two small beakers of sodium azide and lead nitrate solutions must be added very carefully to avoid physical mixing of the two solutions. The dish is covered and left undisturbed for several hours. If left for more than about 6 hours, some transformation to Form I may occur. Figures 5 and 6 show typical crystals of Form II.

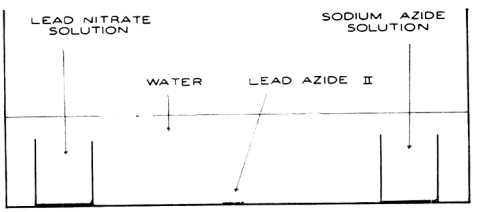

Figure 4. Crystallization dish and small beakers arranged for formation of lead azide II by slow diffusion

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.
Form and Habit. Flattened rods elongated parallel to b showing the basal pinacoid {001}, orthopinacoid {100}, and clinodome {011}.
Axial Ratio. $a:b:c = 2.063:1:0.577$.
Interfacial Angles (Polar). $011 \wedge 0\bar{1}1 = 57° 38'$.
Profile Angle (Observed). 59° 56′ (projection of $011 \wedge 0\bar{1}1$ on 100).
Beta Angle. 107.5°.

X-RAY DIFFRACTION DATA

Space Group Cm, $C2$, or $C2/m$ (*1*).
Cell Dimensions. $a = 18.31$ A.; $b = 8.88$ A.; $c = 5.12$ A. $a = 18.49$ A.; $b = 8.84$ A.; $c = 5.12$ A. (*1*).
Formula Weights per Cell. 8 (8.10 calculated from x-ray data).
Formula Weight. 291.3.
Density. 4.93 [displacement in ethylene dibromide (*2*)]; 4.87 (x-ray).

Principal Lines

d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1
5.40	<1	2.72	<1	1.767	4	1.335	1
5.03	1	2.56	3	1.697	1	1.320	1
4.92	7	2.50	<1	1.677[a]	1	1.306	<1
4.72	<1	2.45	6	1.668[a]	1	1.271	1
4.53	1	2.26	3	1.646	1	1.264	1
4.41	7	2.22	4	1.633	1	1.239	1
4.17	1	2.15	3	1.586	1	1.229	1
				1.569[a]	1		
4.04	1	2.02	1	1.562	1	1.220	1
3.98	9	2.01	5	1.534	1	1.210	1
3.86	<1	1.98[a]	2	1.456[a]	2	1.192	1
3.30	3	1.97[a]	2	1.448[a]	2		
3.12	3	1.92	3	1.410	2		
2.96	<1	1.91	1	1.388[a]	2		
2.91	10	1.84	<1	1.381[a]	2		
2.84	1	1.83	2	1.346	1		

[a] Not well resolved.

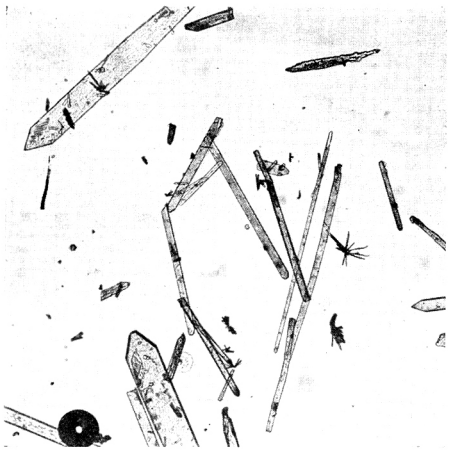

Figure 5. Typical crystals of lead azide II prepared in crystallizing dish shown in Figure 4.

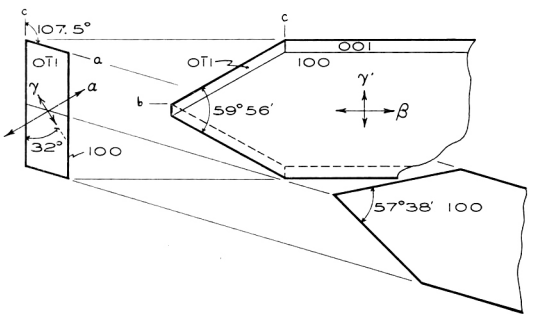

Figure 6. Orthographic projection of typical crystal of lead azide II

OPTICAL PROPERTIES

Refractive Indices (5893 A., 25° C.). $\alpha = 1.98 \pm 0.03$. $\beta = 2.14 \pm 0.01$. $\gamma = 2.7$ (calculated from α, β, and $2V$).

Optic Axial Angles (5893 A.; 25° C.). $2V = 67 \pm 3°$ (estimated from curvature of the brushes).

Dispersion. Inclined dispersion; one isogyre shows $v > r$ and the other isogyre shows $r > v$.

Optic Axial Plane. 010.

Sign of Double Refraction. Positive.

Acute Bisectrix. γ.

Extinction. $\gamma \wedge c = 32°$ in acute β.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 2.26$. R (obsd.) = 34.7.

ACKNOWLEDGMENT

The x-ray powder data on both forms were obtained by Irene Corvin. This program was supported by Contract No. DAI-11-022-ORD-(P)-18 from the Office of the Chief of Ordnance, U. S. Army.

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(1) Azaroff, L. V., *Z. Krist.*, in press.
(2) Miles, F. D., *J. Chem. Soc.* **1931**, II, 2532.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, 500 East 33rd. St., Chicago 16, Ill.

Addendum

SEVERAL serious errors in past descriptions have come to our attention. The most serious of these completely negates the description of 1,2,5-trinitrophthalene I (No. 82 in the series, published in June 1954) as a result of work by E. R. Ward and his associate, J. G. Hawkins, Leicester College of Technology and Commerce, Leicester, England. They have shown by means of infrared absorption that the crystals for which the data were reported are actually a solid solution of 1,3,5- and 1,4,5-trinitronaphthalene. The fact that these two compounds form solid solutions and that crystals of these two isomers in a 1 to 1 solid solution closely resemble the crystals of the reported 1,2,5-trinitronaphthalene has also been confirmed by microscopic fusion methods. The description published is, therefore, not that of 1,2,5-trinitronaphthalene but of a solid solution of the 1,3,5- and 1,4,5- isomers containing a slight excess of the 1,4,5-isomer. Ward also reports that a small amount of the 1,5-dinitronaphthalene may be present.

The second error involves the single crystal x-ray data for 4-aminosalicylic acid published as description 98 in September 1955. Notice of this error came from Harry A. Rose, Lilly Research Laboratories, Eli Lilly and Co., and from Giordano Giacomello and his associates, F. Bertinotti and A. M. Liquori, Instituto di Chimica Farmaceutica and the Centro di Strutturistica Chimica, University of Rome, Rome, Italy. The situation is best described by quoting from a letter received from Giacomello.

We are surprised to see that the unit cell dimensions given by Krc and McCrone, apart from a different (and legitimate) choice of crystallographic axes, show rather large deviations from those reported by us in a paper on the structure of the same compound published in *Acta Crystallographica* [Bertinotti, F., Giacomello, G., Liquori, A. M., *Acta Cryst.* **7**, 808 (1954)].

In fact, with the following index transformation:

$$H = 2h - 1$$

$$L = h$$

the unit cell dimensions reported in our paper:

$$\begin{aligned} a &= 7.28 \text{ A.} \\ b &= 3.82 \text{ A.} \\ c &= 25.33 \text{ A.} \\ \beta &= 103° \end{aligned} \qquad (1)$$

become:

$$\begin{aligned} a &= 26.22 \text{ A.} \\ b &= 3.82 \text{ A.} \\ c &= 7.28 \text{ A.} \\ \beta &= 109.75° \end{aligned} \qquad (2)$$

which are not in agreement with those given in description 98:

$$\begin{aligned} a &= 24.62 \text{ A.} \\ b &= 3.795 \text{ A.} \\ c &= 7.239 \text{ A.} \\ \beta &= 108.6° \end{aligned} \qquad (3)$$

Spacings calculated from dimensions 2 compare very well with those experimentally determined by Krc and McCrone from the x-ray powder photograph, which we have reproduced with the compound crystallized according to the procedure suggested by these authors. It should, however, also be mentioned that the powder data obtained with a high resolution x-ray spectrometer show splitting of some of the lines which are unresolved in the powder photograph taken by Krc and McCrone.

Having ruled out the possibility that the differences between cell dimensions 2 and 3 can be attributed to some kind of polymorphism, for sake of precision in the collection of crystallographic data which is being made by ANALYTICAL CHEMISTRY the data given by Krc and McCrone for 4-aminosalicylic acid should be replaced by either dimension 1 or 2.

Both correspondents also question the melting point of 220° C. given in the original description for 4-aminosalicylic acid. The value of 220° C. is, however, correct, although mention should have been made that this value was obtained on the Kofler hot bar before significant decomposition had occurred (in 3 seconds from the time the sample is placed on the bar). This compound decomposes extremely rapidly, as indicated by the fact that the melting point measured 10 seconds after the sample is placed on the hot bar is already down to 190° C. The figure of 3 seconds chosen is the time required for finely ground crystals of a thermally stable compound to exhibit its known melting point.

W. C. McCrone

SCIENTIFIC COMMUNICATION

Identification of Amines as Tetraphenylborates

SIR: We have found that basic organic nitrogen compounds as well as their salts can be detected by precipitation of their tetraphenylborate compounds in aqueous acid solutions. A simple potentiometric method for determining the tetraphenylborate ion [$(C_6H_5)_4B^-$] contents of these salts has been developed. Such analyses together with melting point values provide a new, rapid way of identifying basic amines.

Sodium tetraphenylmetaborate (Na TPB) is known to precipitate quantitatively potassium and ammonium ions as well as certain alkaloids from aqueous hydrochloric acid. A comprehensive bibliography has been reported recently on this subject by Barnard (*1*). Schultz and Goerner (*9*) found that quantitative determinations were possible with certain alkaloids in the 100-mg. range. Marquardt and Vogg (*6*) showed that the tetraphenylmetaborate derivatives of choline and acetylcholine hydrochloride had sharp melting points, after recrystallization from acetone. Zeidler (*12*) reported that the alkaloids—cadaverine, histamine, histidine, guanidine, putrescine, and spermine—yielded tetraphenylmetaborate precipitates, which possessed rather sharp melting points even without recrystallization.

We have found that this reaction has wide application. Any organic amine, which is basic enough to place a positive charge on its nitrogen atom, can be precipitated with 0.6% sodium tetraphenylmetaborate solution from aqueous solutions of its hydro or quaternary halide salts as its tetraphenylborate derivative. In fact, the results obtained from testing about 120 water- and acid-soluble organic compounds, covering all possible functional group types, indicate that sodium tetraphenylmetaborate can be used to test qualitatively for basic nitrogen compounds. The test is very sensitive as well as specific. In the case of methyl-4-picolinium iodide, a precipitate is detectable at a concentration as low as $10^{-3}M$, after centrifuging. This compound was precipitated quantitatively in the 0.4- to 1.0-mmole range. A study of the stoichiometry of the reaction between sodium tetraphenylmetaborate and other nitrogen compounds is now in progress.

MATERIALS

The sodium tetraphenylborate (reagent grade) was purchased from the J. T. Baker Chemical Co. and used directly.

The potassium tetraphenylborate was prepared according to the procedure recommended by Sporek and Williams (*10*).

Tetra-*n*-butylammonium bromide was prepared according to the procedure of Sadek and Fuoss (*8*). The colorless crystals melted at 118–20° C.

Methylpyridinium iodide, melting point 123–5°, and 1,2-(di-4-pyridyl)-ethane, melting point 114.5–16.0°, were synthesized and recrystallized according to Bergmann, Crane, and Fuoss (*2*).

Methyl-4-picolinium iodide, melting point 157–8°, and 2-iodoethylpyridinium iodide, melting point 108–10°, were prepared according to Crane and Fuoss (*3*).

Tetramethylammonium iodide was prepared by the usual addition of methyl iodide to trimethylamine. Excess alkali was added to an aqueous solution of trimethylamine hydrochloride, and the free amine bubbled into 95% ethanol, immersed in ice water. Half as much volume of cold ether was added, followed by a 50% molar excess of methyl iodide. White crystals soon began to form at 10°. After the reaction had subsided, the vessel plus contents was allowed to stand for 10 hours at 25° After filtration and three 20-ml. washes with cold ether, glistening white crystals, melting above 230°, were obtained.

Methylquinolinium iodide was obtained by the addition of 30 grams of methyl iodide to 15 grams of redistilled quinoline in 40 ml. of ether. The mixture was allowed to stand 24 hours at 25° in the dark in a rubber-stoppered flask. The orange solid was separated by filtration and washed with absolute ether. Recrystallization from benzene yielded yellow crystals, melting at 133°.

A sample of 2,2′-dipyridylamine was synthesized according to Tschitschibabin's procedure (*11*). The starting material, crude 2-aminopyridine, A, was obtained from the Reilley Tar and Chemical Corp. Condensation of equal molar amounts of compound A and its hydrochloride salt (prepared by bubbling anhydrous hydrogen chloride into an ethanol solution of A, followed by evaporation of the solvent) at 240° in the absence of moisture gave a 25% yield of the desired 2,2′-dipyridylamine. Crystallization from hot water gave long white needles, melting at 95°.

Methyldiethylsulfonium iodide was prepared by the addition of a 70% molar excess of methyl iodide to 8 grams of diethyl sulfide in 30

Table I. Compounds Showing No Reaction with Sodium Tetraphenylborate

No.	Compound	Structure Type	No.	Compound	Structure Type
1	Benzyl chloride	Hydrocarbon	26	Citric acid	Acid
2	Methanol	Alcohol	27	Succinic acid	Acid
3	Ethanol	Alcohol	28	*p*-Hydroxybenzoic acid	Acid
4	2-Propanol	Alcohol	29	*p*-Toluenesulfonic acid	Acid
5	2-Methyl-2-propanol	Alcohol	30	Urea	Amide
6	Ethylene glycol	Alcohol	31	Thiourea	Amide
7	Glycerol	Alcohol	32	*n*-Butyl formate	Ester
8	Phenol	Phenol	33	Ethyl acetoacetate	Ester
9	*p*-Nitrophenol	Phenol	34	Dextrose	Sugar
10	1-Naphthol	Phenol	35	Fructose	Sugar
11	Resorcinol	Phenol	36	Lactose	Sugar
12	Hydroquinone	Phenol	37	Maltose	Sugar
13	2,2′-Dihydroxyethyl ether	Ether	38	Sucrose	Sugar
14	Heliotropin	Ether	39	Alizarin sodium sulfonate	Quinone
15	Paraldehyde	Ether	40	Azoxybenzene	Azo
16	Ethyloxonium chloride	Ether	41	Methyl orange	Azo
17	Formaldehyde	Aldehyde	42	Ethanolamine	Amine
18	Chloral hydrate	Dihydroxy	43	Trishydroxymethylaminomethane	Amine
19	Acetone	Ketone	44	Hydroxylamine hydrochloride	Amine
20	Methyl ethyl ketone	Ketone	45	Semicarbazide hydrochloride	Amine
21	Formic acid	Acid	46	*p*-Nitrophenylhydrazine	Amine
22	Acetic acid	Acid	47	2,4-Dinitroaniline	Amine
23	Lactic acid	Acid	48	*d,l*-Alanine	Amino acid
24	Pyruvic Acid	Acid	49	Sulfanilic acid	Amino acid
25	Thioglycolic acid	Acid	50	Sodium sarcosinate	Amino acid

Table II. Compounds Yielding White Precipitates Immediately at 25° with Sodium Tetraphenylborate

No.	Compound	Melting Points of Precipitates, °C.
	A. Quaternary Ammonium Salts	
51	Tetramethylammonium iodide	Above 360
52	Tetrabutylammonium bromide	229–31[a]
53	Tetraethanolammonium hydroxide	160–62
54	Betaine	118–19.5
55	Methylpyridinium iodide	242–3
56	Methyl-4-picolinium iodide	201–4
57	*N*-2-Iodoethylpyridinium iodide	
58	Methylquinolinium iodide	196.5–9.5
	B. Heterocyclic Amines	
59	Pyridine	229–31.5
60	2,2′-Dipyridylamine	179–83
61	Quinoline	135–7
62	8-Quinolinol[c]	199–202[a]
63	1,2-Di-(4-pyridyl)ethane	
64	2-Methylpyrazine	171–8
65	2,5-Dimethylpyrazine	141–4
66	Piperidine	
67	Hexamethylenetetramine	160–1
68	Caffeine	128–9
69	Creatinine	143.0–3.5
	C. Primary Aliphatic Amines	
70	Methylamine hydrochloride	209–11
71	Ethylamine hydrochloride	163–9
72	Butylamine hydrochloride	126.5–8.5
73	Glycine methyl ester	121–3[a]
74	Benzylamine	178–182[a]
75	2-Phenylethylamine	173–5
	D. Secondary Aliphatic Amines	
76	Dimethylamine	157–61
77	Diethylamine	169–70
78	Diisopropylamine	155–6
79	Dibutylamine	144–5
80	Dibenzylamine	129–33
	E. Tertiary Aliphatic Amines	
81	Trimethylamine	170–2
82	Triethylamine	172–4
83	Triethanolamine	143–5
84	Dimethylbenzylamine	182–5
85	Methyldibenzylamine	140–2
	F. Primary Aromatic Amines	
86	Aniline	125.5–8.5
87	*p*-Bromoaniline	92.5–7.5[b]
88	*p*-*tert*-Amylaniline	146–9
89	*p*-Methylaniline	141.5–4
90	*o*-Methylaniline	119–21
91	*o*-Methoxyaniline	123–5
92	1-Naphthylamine[d]	111–13[b]
93	2-Naphthylamine	138.5–40.5[b]
94	*p*-Hydroxyaniline[e]	105–8
95	*o*-Phenylenediamine[f]	140–4
96	*m*-Phenylenediamine	116–21
	G. Secondary Aromatic Amines	
97	*N*-Methylaniline	105–16
98	Phenylhydrazine	144–7[a]
	H. Tertiary Aromatic Amines	
99	*N*,*N*-Dimethylaniline	121–4
100	*p*-Bromodiethylaniline	111–25
101	*p*-Dimethylaminobenzaldehyde	
	I. Quaternary Onium Salts	
102	Methyldiethylsulfonium iodide	177–9
103	*S*-Benzylthiuronium chloride	181–2

[a] Melts repeatedly at this temperature. [b] Compound unstable. [c] Yellow precipitate. [d] Pink precipitate. [e] Brown precipitate. [f] Red precipitate.

Table III. Data Obtained in Potentiometric Standardization of Silver Nitrate with Potassium Tetraphenylborate

(109.8 mg. of K TPB in 100 ml. of 1:1 aqueous acetone, 9.0 mmole each of acetic acid and sodium acetate buffer)

Silver Nitrate, Ml.	*E*, Mv.	ΔV, Ml.	ΔE, Mv.	$\frac{\Delta E}{\Delta V} \times 10^{-2}$	Vol., Ml.
0.000	460				
4.993	498				
5.060	507				
		0.035	13	3.7	5.078
5.095	520				
		0.040	20	5.0	5.115
5.135	540				
		0.020	15	7.5	5.145
5.155	555				
		0.025	35	14.0	5.168
5.180	590				
		0.025	75	30.0	5.193
5.205	665				
		0.030	80	26.7	5.220
5.235	745				
		0.032	70	21.9	5.251
5.267	815				
		0.033	45	13.6	5.284
5.300	860				
		0.030	21	7.0	5.315
5.330	881				

Table IV. Potentiometric Titration of Methylpyridinium-tetraphenylborate with Silver Nitrate

Vol., Ml.	*E*, Mv.	ΔV, Ml.	ΔE, Mv.	$\frac{\Delta E}{\Delta V} \times 10^{-2}$	Vol., Ml.
4.67	810				
		0.05	9	1.8	4.70
4.72	819				
		0.03	7	2.3	4.74
4.75	826				
		0.02	22	11.0	4.76
4.77	848				
		0.02	61	30.5	4.78
4.79	909				
		0.02	176	88.0	4.80
4.81	1085				
		0.02	25	12.5	4.82
4.83	1110				
		0.02	35	17.5	4.84
4.85	1145				
		0.03	25	8.3	4.87
4.88	1170				
		0.04	10	2.5	4.90
4.92	1180				

ml. of ether. After 3 days an orange oil had separated from the cloudy solution. The oil was separated from the ether solution in a separatory funnel and was washed with four 10-ml. portions of ether. Some of the sulfonium iodide, thus obtained, was dissolved in water, without further purification, for testing purposes.

The remaining organic compounds, which were tested, were commercially available products. Whenever necessary, they were purified by recrystallization or simple distillation through a Vigreux column.

PROCEDURE

Amine Detection. The qualitative tests are conducted by dissolving 5 to 10 mg. of the organic compound in water or 6*N* hydrochloric acid, adjusting the pH at 2 to 3, and then diluting to approximately 5 ml. A 0.6% aqueous solution of sodium tetraphenylborate is prepared according to the procedure of Gloss (*4*). A small portion (1 to 2 ml.) of the latter solution is added to the 5 ml. of the former. The immediate formation of a dense, usually white precipitate indicates the presence of a salt of a basic amine.

Amine Identification. For melting point and analysis studies, the borate solution is added slowly with stirring to a 10 to 20% excess (ca. 0.2 gram) of the amine dissolved in 15 to 20 ml. of hydrochloric acid solution, which has been adjusted to a pH of 2 to 3. The tetraphenylmetaborate precipitate is removed by suction filtration, washed well with distilled water, and dried below 60°. A melting point of the tetraphenylmetaborate derivative serves to identify the original amine. A Purdue melting point tube, filled with a high boiling petroleum oil, was used in this laboratory.

The tetraphenylborate derivatives were analyzed for nitrogen by the conventional Dumas method; and for tetraphenylmetaborate ion content by a new potentiometric method, which is based on the insolubility of silver tetraphenylmetaborate in aqueous acetone. Two recent methods for determining potassium are also based on this fact. Rüdorff and Zannier (*7*) added a known excess of 0.05*N* silver nitrate to the potassium tetraphenylmetaborate in acetone, and back-titrated with thiocyanate according to the Volhard procedure. Hahn (*5*) titrated the potassium ion in aqueous solution directly with standard silver nitrate using chromate indicator via the Mohr method.

Our potentiometric method consists of dissolving the tetraphenylmetaborate samples (0.2 to 0.4 meq.) in ca. 90 ml. of 1 to 1 aqueous acetone. A mixture of 3.0 ml. of 3*M* acetic acid and 3.0 ml. of 3*M* sodium acetate is added to buffer the solution. The resulting solution is then titrated with 0.06*N* aqueous silver nitrate, using a Beckman pH meter, a silver wire indicator, and a glass reference electrode to measure the e.m.f. changes. The silver nitrate is standardized against potassium tetraphenylborate which has been reprecipitated from acetone.

DISCUSSION

The immediate formation at 25° of a dense, usually white, precipitate, upon addition of the tetraphenylborate ion to an aqueous solution, establishes the presence of a basic amine ion, providing certain interfering cations are known to be absent. The inorganic cations—potassium, rubidium, cesium, ammonium, and mercuric ions—also yield precipitates (*4*) under the conditions of the test. As Table I shows, sodium tetraphenylborate is without effect on uncharged organic compounds. Of all cations (see Table II) that gave a positive test, only two contained their

Table V. Comparative Slopes in Potentiometric Titration of Tetraphenylborate Salts with Silver Nitrate

Compound	Slope, Max.	Equiv. Pt., Ml.
Methylpyridinium TPB	88.0	4.800
Diethylamine TPB	62.5	5.635
Glycine methyl ester TPB	40.0	6.058
Sodium tetraphenylborate	35.0	
Hexamethylenetetramine TPB	33.3	4.546
Potassium tetraphenylborate	30.0	5.213
Anilinium tetraphenylborate	4.3	

Table VI. Analysis of Tetraphenylborate Derivatives

Original Compound	Tetraphenylborates M.p., ° C.[a]	$(C_6H_5)_4B$, % Calcd.	Found	Nitrogen, % Calcd.	Found
Tetraethanolammonium hydroxide	160–2	61.7	60.7	2.69	2.73
Tetra-*n*-butylammonium bromide	229–31	56.0	55.9	...	...
Betaine	117–8.5	73.1	73.8	3.21	3.35
Methylquinolinium iodide	196–9	68.9	69.8	2.96	3.05
Hexamethylenetetramine	160–1	69.3	69.5	12.16	12.09
Trimethylamine	170–2	84.2	82.6	3.70	3.69
Diethylamine	169–70	81.2	80.6	3.57	3.71
Methylamine	209–11	90.9	89.5	4.00	4.52
Aniline	126–9	78.0	[b]	3.42	4.33

[a] Corrected values.
[b] Slope so poor that no definite end point was detected. This is generally true for primary aromatic amines.

positive charge on an atom other than nitrogen. These interfering onium salts are methyldiethylsulfonium iodide and *S*-benzylthiuronium chloride.

As Table II indicates, all possible types of basic amines yield precipitates. The size or shape of the cation does not appear to be a critical factor. The general reaction can be formulated:

$$\text{Org N}^+ + \text{B}(\text{C}_6\text{H}_5)_4^- \rightarrow \text{Org NB}(\text{C}_6\text{H}_5)_4 \downarrow$$

where Org N^+ can be an ammonium ion in any of the four possible stages of substitution—i.e., RNH_3^+, $RR^1NH_2^+$, $RR^1R^2NH^+$, or $RR^1R^2R^3N^+$. The radicals, R through R^3, may be alike or dissimilar. The results also show that only amines, whose basic ionization constant is at least as great as 1×10^{-11}, can yield precipitates immediately at 25°. For example, aniline hydrochloride reacts immediately, whereas the introduction of one negative nitro group in the benzene ring causes *p*-nitroaniline to react so slowly that only a few crystals of the tetraphenylborate derivative form after long standing. When two nitro groups are present, as in 2,4-dinitroaniline, no precipitate whatever is formed. The presence of solubilizing groups in the amine ion also hinders the formation of a precipitate. Only small quantities of tetraphenylborate precipitate are obtained from large amounts of *p*-hydroxyaniline; and the solubility product was not even exceeded in the cases of ethanolamine and trishydroxymethylaminomethane. Because triethanolamine is of higher molecular weight, it is readily precipitated. The introduction of a methyl group into the structure of glycine to form its methyl ester (compound 73 of Table II) was found to decrease the solubility of the tetraphenylborate derivative enormously. Whereas three amino acids (Nos. 48, 49, and 50 of Table I) gave no precipitates under any conditions, *p*-aminobenzoic acid, *l*-proline, *l*-tyrosine, *dl*-serine, *dl*-aspartic acid, and *l*-cysteine hydrochloride will yield white precipitates, if the solution is heated.

In Table II are recorded the melting points of various tetraphenylborate derivatives. While most of these derivatives melt with decomposition, the ranges are sharp enough and of a suitable temperature (somewhere between 90° and 240°) to identify the amine. Although the original tetraphenylborate precipitates need not be recrystallized or purified further for identification purposes, some can be recrystallized from aqueous methanol or acetone. However, several underwent decomposition when warmed in these solvents. Thus, because they are so easily prepared and do not need to be recrystallized, tetraphenylborates are superior to other derivatives commonly used to identify basic nitrogen compounds. Also, the melting points of these tetraphenylborate derivatives are indicative of and sensitive to slight structure variations. For example, introduction of a *p*-methyl group on the pyridine ring (see compounds 55 and 56 of Table II) lowers the melting point about 40°. Placement of an 8-hydroxy group on the quinoline nucleus (see compounds 61 and 62) raises the melting point 64°. As illustrated by compounds 70 through 72, an increase in the length of the carbon chain is accompanied by a distinct drop in the melting point. The sensitive effect of the position of a substituent group on the melting point of an aromatic amine is exhibited by compounds 89, 90, 92, 93, 95, and 96.

The potentiometric determination of tetraphenylborate ion is based on the stoichiometric completeness of the reaction:

$$\text{Ag}^+ + (\text{C}_6\text{H}_5)_4\text{B}^- \rightarrow \text{AgB}(\text{C}_6\text{H}_5)_4 \downarrow$$

in 1 to 1 aqueous acetone solution. Typical data obtained in titrations with aqueous silver nitrate solution are listed in Tables III and IV. The column headings stand for the following: Vol. is milliliters of silver nitrate solution added; E is the electromotive force in millivolts; ΔV is the increment of volume; ΔE is the increment of electromotive force; and $\Delta E/\Delta V$ is the rate of change of E with silver nitrate added. When the values of $\Delta E/\Delta V \times 10^{-2}$ are plotted against silver nitrate added, the maxima of the graphs locate the equivalence points.

Figure 1 shows the differential curves obtained by titrating some representative tetraphenylborate derivatives with silver nitrate solution: hexamethylenetetramine, methylpyridinium, potassium, diethylamine, and glycine methyl ester. The maximum values of the slopes obtained experimentally for these deriva-

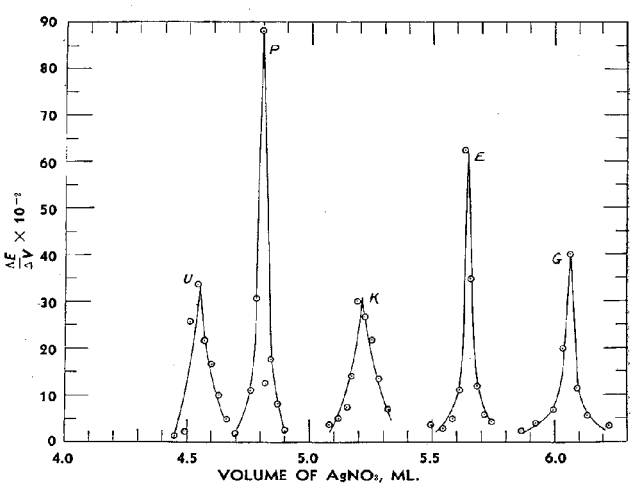

Figure 1. Differential potentiometric plots of titration of tetraphenylborates with silver nitrate

U. Hexamethylenetetramine
P. Methylpyridinium
K. Potassium
E. Diethylamine
G. Glycine methyl ester

tives as well as for sodium tetraphenylborate and anilinium tetraphenylborate are recorded in Table V, which also lists the estimated equivalence points. These data indicate that the slopes at the equivalence point are greatest for the heterocyclic and aliphatic amine borates, and steep enough to make possible the detection of an end point for all other types except the primary aromatics (represented by the anilinium derivative).

The nitrogen and tetraphenylborate analyses for a series of representative derivatives are listed in Table VI. An examination of the data indicates that only one tetraphenylborate group adds per molecule of amine, regardless of the number of nitrogen atoms present. For some 25 compounds analyzed, the values for tetraphenylborate found were within 3% (relative) of theory. These values were reproducible to a relative precision of 0.3%.

ACKNOWLEDGMENT

This work was made possible by a Cottrell grant from the Research Corp., New York, N. Y. and by a grant from the Rutgers University Research Council. The author thanks Eva Agnes Smith for assistance in the experimental work.

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RECEIVED for review March 6, 1956. Accepted August 6, 1956. Presented in part before the Analytical Division, Meeting-in-Miniature, North Jersey Section, ACS, South Orange, N. J., January 30, 1956.

MEETING REPORT

Third Ottawa Symposium on Applied Spectroscopy

THE Third Ottawa Symposium on Applied Spectroscopy was held September 12 to 14 in Ottawa under the auspices of the Ottawa Valley Section of the Canadian Association for Applied Spectroscopy, with the cooperation of the Mines Branch and the Geological Survey Branch, Department of Mines and Technical Surveys. Abstracts of the technical papers presented are given here.

Spectrochemical Analysis of Lead Alloys. J. H. D. HOWARTH AND H. NAUSESTER, Canada Metal Co., Ltd., Toronto, Ontario.

The behavior of various alloying elements under a number of difficult analytical conditions has been studied. Some of the problems of this type of analysis were discussed and a suggested method of analysis was outlined.

Spectrochemical Analysis of Corrosion Products on Lead Sheath of Cables. W. J. BENNETT, Northern Electric Co., Ltd., Lachine, Quebec.

Products adhering to lead sheaths, usually in small amounts, were analyzed to determine major and minor components. The method utilizes a mixture of sample, germanium dioxide, and graphite powder, which is pelletized. A critically damped multisource discharge is used initially to spark the pellet and the resultant spectrum is recorded. The pellet is then arced with a heavily overdamped discharge and the resultant spectrum is separately recorded. Densitometered spectral lines are used to prepare working curves for the individual elements sought.

Generalized Working Curves for Both High- and Low-Alloy Steels. L. O. EIKREM, Baird Associates–Atomic Instrument Co., Cambridge, Mass.

Both high- and low-alloy steels may be analyzed from the same analytical working curves when atomic dilution theory is properly applied. This procedure assists considerably in establishing working curves, inasmuch as separate sets of standards for each alloy type need not be prepared. The paper demonstrates the establishment of generalized working curves by use of the $(M + X)$ factor previously reported. M is the concentration of the matrix element, and X is the concentration of the element being analyzed. Points obtained with standards are translated vertically to a constant $(M + X)$ value in accordance with the equation:

$$X' = X\,(M' + X')/(M + X)$$

Curves for the various elements need not be plotted at the same $(M + X)$ value. The concentration values, X', for the various elements as read from the curves are used to solve for actual concentrations, X, using the formula:

$$X_i = \frac{100\ x_i'}{1 + \sum_1^n x_i'}$$

in which

$$x_i' = X_i'/(K_i' - X_i')$$

K_i' represents the value of $(M + X)$ for the element i curve. The calculations are quickly made with the help of appropriate tables and a calculating machine. When a large number of analyses of the same alloy type must be made, individual curves for each type may be set up to read actual concentration directly, although the standards used in setting up these curves may represent a variety of alloy types.

Comparative direct reader and wet chemical results were shown for a number of high-alloy types, including stainless. Coefficients of variation of 1% or better of the amount present between direct reader and chemical values for the high concentration elements are readily obtainable.

Spectrographic Sample Forms in Use at a Copper and Brass Mill. P. R. DE LHORBO, Anaconda American Brass, Ltd., New Toronto, Ontario.

Sample forms have been developed to meet changing requirements of casting shop, mill, and miscellaneous materials such as scrap and fabricated parts. An analytical procedure using a laboratory cast pin sample was described in detail.

Spectrographic Determination of Impurities in Selenium. N. TOMINGAS AND W. C. COOPER, Canadian Copper Refineries, Ltd., Montreal, Quebec.

Rapid and accurate spectrochemical procedures for the estimation of impurities in refined and high purity selenium were described. In the analysis of high purity selenium external comparison standards, which have been evaluated by accurate chemical methods, are employed. The use of such standards permits the rapid routine analysis of large numbers of samples. Mercury and tellurium in refined selenium are estimated densitometrically. A split filter of 10 and 100% transmission enables the use of selenium as internal standard and the determination of tellurium in the range 0.01 to 1%. The agreement with chemical analyses is excellent.

Segregation Studies in Titanium Alloys by the Spectrographic Microvolume Technique. J. K. HURWITZ, Mines Branch, Ottawa, Ontario.

Segregation occurs in titanium alloys containing 4% manganese and 4% aluminum as well as 6% aluminum and 4% vanadium. Samples of these alloys were analyzed by the spectrographic microvolume technique and major segregation of the alloying constituents was found.

Standards for Spectrographic Analysis of Magnesium and Its Alloys. L. R. PITTWELL, Dominion Magnesium, Ltd., Haley, Ontario.

The dangers of indiscriminate use of purchased standards arise from variation in shape between standard and sample and differ-

ences in preparation. The fundamental requirements of a good standard are: homogeneity, enough material, shape, and accurate analysis. Of the methods of preparation, chill casting and extrusion from a homogeneous billet are the best. Details of preparation and analysis were given.

A High-Resolution Grating Flame Spectrometer. R. W. TABELING AND R. K. BREHM, Jarrell-Ash Co., Newtonville, Mass.

A high-resolution grating flame spectrometer was described. The increase in sensitivity, precision, and scope of analysis obtainable with this apparatus was described and substantiating data were presented.

Introducing Spectroscopy into Sophomore Analytical Chemistry. F. C. STRONG III, Stevens Institute of Technology, Hoboken, N. J.

Surveys of analytical literature and studies of practices at several large consulting analytical laboratories show that spectrochemical methods, both emission and absorption, are used more than any other methods. Yet most current introductory analytical courses and textbooks pay little attention to them.

Elementary but instructive spectrochemical analyses have been introduced into the sophomore course at Stevens. Using visual spectrometers, a qualitative flame technique has been devised that gives more persistent emission than is obtained by using a drop of solution on a platinum wire. Qualitative analysis by sparking solutions in the Spectranal and home-made devices imitating it have been successful.

While line readings were taken visually, experiments are under way to apply the Land-Polaroid camera to a spectrometer, permitting rapid spectrography without a dark room. Quantitative emission spectroscopy has not been tried yet at Stevens, but a dilution technique has been published.

In contrast with emission, absorption spectroscopy with simple visual equipment is more successful in quantitative than in qualitative analysis. Absorption maxima can be demonstrated for permanganate ion, using a wedge-shaped cell and adjusting to an optimum thickness, but no other common colored inorganic ion seems to have a maximum in the visible range. On the quantitative side, approximate results can be obtained visually by matching solutions in test tubes. Using a photoelectric colorimeter and, with a working curve prepared, a large number of students can measure their samples on one instrument in a short time.

Spectrographic Determination of Trace Impurities in Uranium and Thorium. W. D. MACKINTOSH AND L. W. WRAY, Atomic Energy of Canada, Ltd., Chalk River, Ontario.

Excitation of a matrix of uranium or thorium results in a spectrum in which it is impossible to distinguish the spectral lines of any trace impurities. This paper describes a method for separating the impurities from the bulk of the matrix, so that their spectra can be examined free of the obliterating effect of the uranium or thorium spectra.

The uranium or thorium is extracted from an aqueous solution of the sample by means of an organic complexing agent. Conditions necessary to obtain adequate decontamination have been established. Following extraction, the aqueous layer containing the impurities is concentrated and an aliquot deposited on a graphite electrode for excitation by condensed alternating current sparking.

Two methods of correcting for the mechanical loss in the aqueous fraction invariably encountered in aqueous-organic separation have been used. In the purely spectrographic method a known amount of cobalt is added to the solution before extraction and the amount carried through to the electrodes is determined from examination of a selected line. The loss factor is applied to the calculations of the amounts of all the impurities. If cobalt is present in the sample, active cobalt-60 in a quantity below the limits of spectrographic detection is added and is counted before and after extraction to establish the factor.

Spectrochemical Analysis of Radioactive Minerals. W. O. TAYLOR, Ontario Department of Mines, Toronto, Ontario.

In a study of radioactive mineral occurrences in the Bancroft, Ontario, area, the spectrochemical analysis of selected mineral grains of several different minerals from different occurrences was undertaken. Up to 20 constituents were determined in each mineral on as little as 2 mg. of sample. Techniques used, difficulties encountered, and results obtained were shown.

Quantitative Spectrographic Determination of Lithium in Thorium Metal and Compounds. P. S. HARDY, Atomic Energy of Canada, Ltd., Chalk River, Ontario.

A spectrochemical procedure for the determination of lithium in thorium for the range 0.2 to 2.0 p.p.m. has been developed. The method of excitation used was on alternating current arc, with graphite electrodes. The preparation of samples and standards, the spectral lines chosen as analysis and internal standard lines, and the limit of detection and accuracy of the method were described.

Determination of Precious Metals in Ores. C. L. LEWIS, Falconbridge Nickel Mines, Ltd., Richvale, Ontario.

In an analytical project conducted by Falconbridge Nickel Mines, Ltd., precious metal determinations made by commercial analysts on the same samples varied over a wide range. Assuming the variations to be due to differences in fire assay technique, an experiment was designed to find a technique which would provide maximum precious metal collection and would allow other assayers to reproduce the results obtained. Spectrographic analyses of fire assay beads from four laboratories showed much smaller variations than those found in the earlier project. As all spectrographic analyses were made by the same method in one laboratory, it is probable that analytical technique rather than fire assay technique was responsible for the earlier variations.

Within the range of variations found in this work, one fire assay technique gave slightly higher platinum results than those of other laboratories and, in most cases, comparable palladium results. No other significant trends were noted, except that gold is apparently a better collector for rhodium than is silver. The fire assay technique which gave higher platinum values was tested in one of the other laboratories; values obtained were comparable to the original results.

Some Factors in the Preparation and Use of Nonmetallic Standard Samples. J. E. BURGENER, Technical Service Laboratories, Toronto, Ontario.

Some factors involved in preparing standard samples for spectrographic analysis of solutions, powders, metals, filings, etc., were discussed.

Spectrochemical Analysis of Steel Plant Materials. J. H. KELLY, Steel Co. of Canada, Ltd., Hamilton, Ontario.

Optical emission spectrography has been applied to the analysis of the raw materials and products of the steel-making processes. Some of the routine methods used for control purposes were described. The economic utilization of both optical emission and x-ray emission techniques was discussed, with reference to the applications which arise within the steel plant.

Complementary Nature of X-Ray Fluorescence Methods and Optical Emission Methods in Spectrochemical Analysis. B. R. BOYD AND M. F. HASLER, Applied Research Laboratories, Glendale, Calif.

The limits in accuracy, sensitivity, and elements detected which can be achieved by optical emission methods were compared to those attainable by x-ray methods. A comparison of the sampling requirements of each method was considered.

On this basis, various analytical problems were discussed, and the complementary nature of x-ray and optical emission methods was shown. These problems cover examples in analyses of complex metal alloys, ore and slag, cement, glass, chemicals, and petrochemicals. In order to allow laboratories to utilize the two methods most effectively, instrumentation of complementary design was described, and some practical results were shown. An x-ray instrument suitable for continuous analysis of a single element was likewise described, and its place in the field of automation was surveyed.

X-Ray Emission Spectrography Applied to Analysis of Geological Material. G. R. WEBBER, Department of Geological Sciences, McGill University, Montreal, Quebec.

X-ray emission spectrography is being applied to the rapid analysis of rocks and ores in the Department of Geological Sciences at McGill University. Various techniques of analysis, such as internal standard, addition, and dilution methods have been applied to determination of such elements as niobium, uranium, calcium, titanium, and potassium. Throughout the course of the work there have been advances in commercial equipment available for x-ray emission spectrography, and many of these advances have been applied in our laboratory. We have made use of a variety of analyzing crystals, including mica, quartz, sodium chloride, lithium fluoride, and EDT crystals. A helium atmosphere and flow counter have been used for detection of soft x-rays. This combination permits detection of elements down to aluminum in atomic number.

Some Applications of X-Ray Fluorescence Techniques to the Analysis of Mine Exploration Samples. E. J. BROOKER, X-Ray Assay Laboratories, Toronto, Ontario.

The analysis of niobium and uranium ores was discussed. A general outline of qualitative and semiquantitative analytical methods as applied to mine exploration work was given.

Versatile Droplet Counter

Grant C. Riggle and Laurence R. Crisp, Department of Health, Education, and Welfare, National Institutes of Health, Division of Research Services, Laboratory Aids Branch, Instrument Section, Bethesda 14, Md.

RESEARCH scientists in the fields of chromatography (*3*) and protein fractionation (*2*) require a reliable and versatile type of electronic detector to count the drops falling from a fractionating column. These requirements led to the development of a droplet counter which, after extensive testing and use, has proved reliable in operation, simple to construct, and readily adaptable to use with existing commercial apparatus. Commercial droplet counters are available, but their circuitry and optical design limit their adaptability for a variety of laboratory research problems.

Most of the previously described photoelectric counters have used intense light sources for activation of the phototube. Heat radiation from such sources causes changes in the chemical structure of some solvents, and partial evaporation of slow-forming drops. Another factor which previously caused difficulty is the necessity for exact balance of the photoelectric circuit. These sources of error have been eliminated by the use of a new photo detection tube and the development of a sensitive electronic circuit.

Features of Developed Model. The photoconductive tube requires very low illumination; no lens system is needed, thus eliminating the focal point adjustment for the interruption of the light beam; only partial blanking of the target tube window by the falling drop is necessary to produce a sufficient signal for counting; phototube blanking time can be as short as 0.5 millisecond; droplet guide tube is designed for easy removal and cleaning to prevent contamination; line voltage fluctuations produce no spurious counts; and the counter is adaptable to existing commercial fraction collectors without modifications. Reliable operation in refrigeration rooms is achieved through the use of heaters in the phototube and the counter enclosures. It was found best to leave the counter energized at all times while in refrigerated spaces, whether in use or not.

Figure 1 shows the developed circuit.

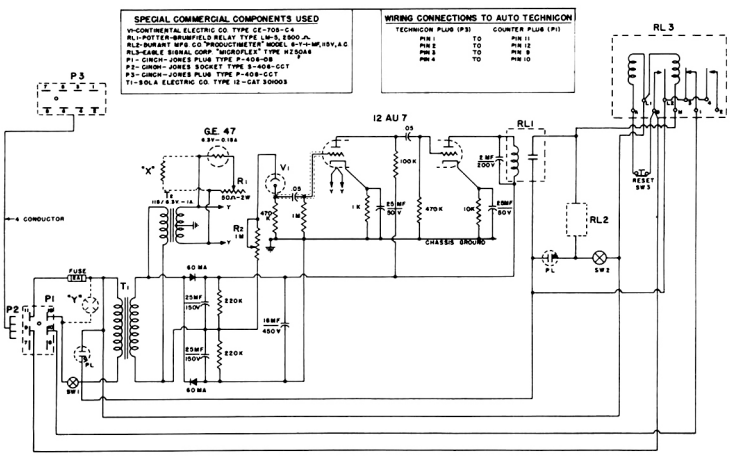

Figure 1. Diagram of circuit

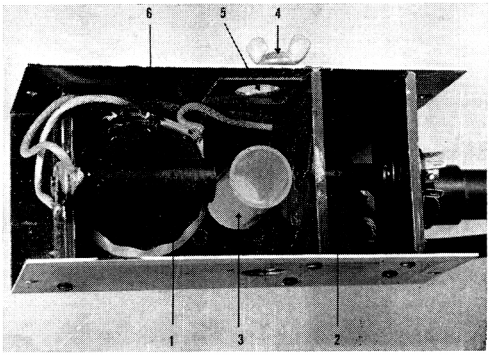

Figure 2. Photoconductive cell housing assembly

The target lamp voltage is varied by the rheostat, R_1, permitting the target lamp to be dimmed to any desired intensity level. Constant voltage transformer, T_1, smoothes out line fluctuations to critical circuits. A selenium-type voltage doubler rectifier keeps the load requirements within the rating of the constant voltage transformer, reduces the chassis space requirements, and provides an inexpensive voltage divider network for supplying stable voltage to the phototube. The photoconductive tube V_1 dark current resistance is in the order of 2.5 megohms and is extremely sensitive to small changes in light. The tube is most responsive to changes in the red-infrared portions of the spectrum. The range of sensitivity is controlled by means of potentiometer R_2. The pulse is amplified through the flip-flop circuit sufficiently to cause relay RL_1 to close its contacts, thus actuating counter RL_2 and relay RL_3. Contacts 1-3 and 2-4 on relay RL_3 may be arranged to operate in a number of sequences as selected by a mechanical adjustment on the relay. When the counter is used with the Technicon fraction collector, the contacts of RL_3 should be set to operate in sequence arrangement No. 1, described in the relay manufacturer's literature. The counter is also readily adaptable to operate with the Gilson and Rinco fraction collectors. A manual reset type count register, RL_2, is provided to totalize the number of counts. Switch SW_3 permits count selector RL_3 to be reset to zero, at the same time advancing the fraction collector one position.

The photoconductive cell housing assembly is shown in Figure 2.

The light source, 1, and the phototube, 2, are covered with light-excluding shields; the droplets fall through glass tube 3, held in place by clamp 4. Windows, 5, are drilled in the housing to permit viewing when operational setups are made. Rheostat 6 controls the voltage to the light source lamp. Resistor X (not shown in this view) is located in the phototube part of the housing. It consists of 2 feet of insulated No. 36 Nichrome wire wound in coil form. This, along with Y, a 25-watt incandescent lamp mounted in the counter enclosure, is necessary only when the counter is used in refrigerated areas.

Maintenance on the units has been negligible. Some counters have been in use 4 years and only the vacuum tube required replacement. The counter has been adapted to use with a new fraction collector (*1*), which permits the collector to be used either as a timed or count-controlled instrument.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Vincent T. Almasy and S. Meredith Meyers, electronic technicians, in the development of the circuit.

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Feed Mechanism for Automatically Capping Fraction-Cutter Tubes

J. G. Kirchner[1] and W. L. Stanley, Fruit and Vegetable Chemistry Laboratory, Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Pasadena, Calif.

IN OPERATING automatic fraction cutters in which volatile materials and substances sensitive to oxidation are collected in moving banks of test tubes, it is desirable to provide a system for capping the tubes as they are filled. During a study of the volatile constituents of citrus juices, an automatic mechanism employing glass balls was developed for capping volatile fractions in test tubes collected from fractional distillations and elution chromatograms.

A schematic diagram of the device is shown in Figure 1, and a three-dimensional diagram is shown in Figure 2.

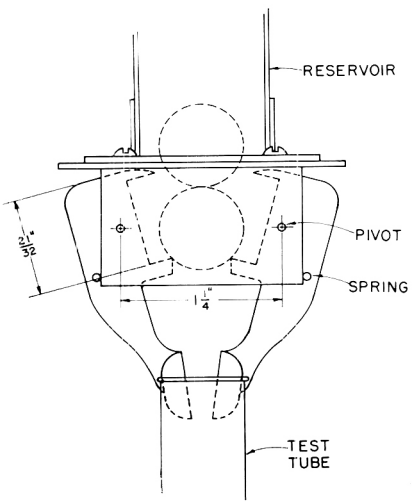

Figure 1. Schematic diagram

The device consists of a storage reservoir connected from above to a chamber in which a marble is held on suspension points by two spring-loaded, pivoted metal jaws. The pivoting jaws are opened and a marble is released when a test tube in the moving rack passes between the lower extensions of the jaws. As the marble is being released, blocking suspension points at the opposite end of the jaws retain the marbles in the reservoir until the capped test tube has moved on. The jaws are then closed by the loading springs, causing the suspension points at the bottom of the chamber to open and release another ball from the reservoir into the feed chamber.

A reservoir for coin-operated candy vending machines, or a long tube, can be used to supply marbles to the feeding mechanism. A simple cone-shaped reservoir cannot be used, because the marbles pile up at the mouth of the cone and block off the opening. However, if a tube is used for the reservoir, it must be bent with an offset, so as to limit to three the number of marbles resting directly on the spring-loaded suspension points.

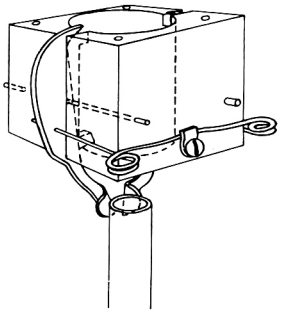

Figure 2. Three-dimensional diagram

Marbles having a diameter of 0.75 ± 0.01 inch are handled satisfactorily by this feeding mechanism. For marbles of this size the space between the suspension points should be $^{21}/_{32}$ inch, as indicated in Figure 1.

Minor variations in the height and diameter of the test tubes do not affect the operation of the feed mechanism. Absence of tubes in the moving bank of collection tubes will not result in the complete discharge of marbles from the reservoir.

After the dropping mechanism had been perfected, the next step was to test the efficiency of differently treated marble-capped tubes to use for maximum retention of low-boiling solvents over extended periods of time.

This was accomplished by setting up two sets each of five untreated tubes, one set of five tubes with greased lips, one set with beveled lips, and one set with beveled-greased lips. Beveling consisted of grinding the tube lips with water and a medium weight Carborundum powder, using one of the glass marbles. The marble was carefully rotated so as to obtain a narrow bevel approximately 1 mm. wide around the inside of the tube lip. As long as the bevel around the lip is unbroken, small variations in tube roundness are insignificant. The beveled rims of one set of tubes were coated with grease. For hydrocarbon solvents, a water-soluble grease similar to that described by Meloche and Frederick [*J. Am. Chem. Soc.* **54**, 3265 (1932)] is satisfactory.

Fifteen-milliliter portions of petroleum ether (boiling range 35° to 60° C.) were next pipetted into all tubes. One set of untreated tubes was stoppered with corks, the remaining tubes were capped with marbles, and all tubes were immediately weighed. The corks were removed from the one set of tubes, and these open tubes and the capped tubes were allowed to stand at room temperature (25° to 27° C.) for 26 hours. After this period the open tubes were again stoppered, and all tubes were reweighed. Efficiency of solvent retention was calculated by subtracting from 100 the product of 100 times the average weight loss from each set of five open tubes.

Table I. Efficiency of Solvent Retention by Ball-Capped Test Tubes

Treatment of Tube Lip	Average Weight Loss, Grams: Open tube, A	Average Weight Loss, Grams: Capped tube, B	Retention Efficiency, $100 - \frac{100B}{A}$
None	5.47	3.48	37
Beveled	5.47	2.92	47
Greased	5.07	1.01	80
Beveled-greased	5.07	0.12	98

Table I indicates maximum solvent retention when beveled-greased test tubes were used. Similar results were obtained with other organic solvents.

ACKNOWLEDGMENT

The authors' appreciation is extended to L. F. Atkinson of this laboratory, who contributed to the design and constructed this apparatus.

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Autofiller

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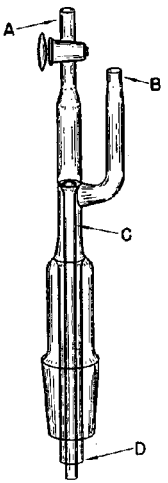
ROUTINE laboratory operations frequently require withdrawing small volumes of previously prepared solutions from large storage containers. To facilitate this operation, an automatic filler utilizing the siphon principle has been designed and fabricated at the Goodyear Atomic Corp. laboratories.



This filler, with the exception of the ground-glass joint and center filler-tube, *C*, is constructed of heavy-walled tubing to minimize breakage. Filler-tube *C* and tube *D* should extend approximately 1 inch beyond the lower end of the ground-glass joint.

The delivery tube from the storage container is attached to arm *A* on the filler by plastic tubing. A similar piece of tubing must be attached to arm *B* and extended above the liquid level of the storage container (a ball check valve in arm *B* may be substituted for the tubing). The filler is placed in a withdrawal bottle fitted with a ground-glass joint. The stopcock is opened and the siphoning action started by means of a rubber squeeze bulb attached to the storage container.

The liquid level in the withdrawal container will cease to rise when the solution reaches annulus *D*. However, the solution will continue to rise in arm *B* until it attains the level of the solution in the storage container (or until the ball check valve is closed). To change withdrawal bottles, it is necessary only to close the stopcock, slowly lift the filler, and allow the solution contained in arm *B* to drain into the bottle, then insert the filler into an empty withdrawal bottle. (Sufficient room will remain in the bottle for the solution contained in arm *B*, if tubes *C* and *D* extend 1 inch beyond the ground-glass joint.)

The ground-glass joint may be of the outer type, which would minimize the danger of pouring the solution over a greased surface.

Work performed under Contract-AT-(33-2)-1, U. S. Atomic Energy Commission.

Gelatin Capsules for Potassium Bromide Infrared Technique

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THE potassium bromide pressed pellet technique has become very useful in infrared spectroscopy, as it avoids many difficulties usually encountered in handling solid samples by other standard procedures [Stimson, M. M., *J. Am. Chem. Soc.* **74**, 1805 (1952)]. The amount of sample and potassium bromide that is employed depends upon the type and size of die available and may be measured by either weighing or approximating the amount by eye. The weighing technique has merit in precise quantitative techniques; however, it is very time-consuming and the potassium bromide both on the sample pan of the balance and in the reagent bottle absorbs moisture during the process. (The potassium bromide in the reagent bottle is lumpy after exposure to the atmosphere, and has an excessively strong 3-micron water absorption band.) The approximation method is of value only in rough qualitative determinations.

A technique which is efficient and virtually eliminates moisture contamination is based upon measuring the potassium bromide as a function of volume rather than weight [described in detail by Mitzner, B. M., *Appl. Spectroscopy* **10**, 75 (No. 2, 1956)], and providing for the storage of predetermined amounts of potassium bromide in individual containers in order to guarantee its purity.

Gelatin capsules of various volumes are readily obtainable, and a size can be chosen that will be appropriate for the particular type of potassium bromide die employed.

In practice, 200-mesh potassium bromide (obtainable from the Harshaw Chemical Co.) is carefully dried at 150° C. (vacuum drying is recommended). The longer portion of the gelatin capsule is filled with the dried potassium bromide, and is occasionally gently tapped in order to ensure an even distribution of the material. Any excess is wiped off and the gelatin cover is put on. The filling procedure should be carried out in a "dry box," or in an air-conditioned room on a dry day.

A large number of capsules can be filled at one sitting, to supply the needs of the infrared spectroscopist for several months. The filled capsules should be placed in an airtight jar or desiccator for future use. When needed, the capsule is opened and its dry contents are emptied into a mixing device along with the sample under investigation.

The volume of the gelatin capsules is very constant. If the capsule is carefully filled, amounts of potassium bromide are reproducible within 2%, which is satisfactory for most quantitative and qualitative infrared procedures.

Where great precision is required, the potassium bromide should be weighed, as the time of tapping during filling can make a difference in the weight of the reagent. This difficulty can be overcome by weighing the empty capsule and afterwards the filled capsule, thus establishing the true weight of the potassium bromide. (The potassium bromide is not exposed to atmospheric contamination during weighing.)

The procedure described enables the analyst to have known amounts of potassium bromide readily available and in a very pure state. Because of the low water content ensured by this technique, highly transparent potassium bromide pellets are obtainable. A further application of this procedure is for micro samples. In order to prevent loss of small quantities of sample, they may be added directly to potassium bromide contained in an oversized capsule and the two components mixed in the capsule with the aid of a mechanical vibrator. The contents can then be emptied directly into the potassium bromide die with a minimum loss of material.

Determination of Ozone by Thermal Conductivity

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IN THIS laboratory, there was a need for rapidly detecting small changes in the ozone concentration of an oxygen stream. Although an electrometric (*3*) and several spectrophotometric (*2, 4*) methods are described, there seem to be no published data on the use of thermal conductivity differences for the determination. Inasmuch as this approach seemed most convenient, its applicability was investigated. The preliminary data are presented in this communication, for this aspect of the problem has been discontinued.

The difference in thermal conductivity between the reference gas (oxygen) and ozonized oxygen of known composition was used to prepare a calibration curve (millivolts *vs.* ozone concentration). The important considerations in obtaining reproducible data are maintaining (1) a constant gas flow through the arms, for a 50% increase in the flow rate caused an 8% error in the concentration determination, and (2) a draft-free and temperature-constant environment. When the cell was sheathed

with glass wool, as the manufacturer recommends, and the temperature of the system did not vary more than 2° C, the zero balance was easily maintained.

While accuracy within 2% of the standard iodometric method was obtained in the concentration range of 0.5 to 1.6 mmoles of ozone per liter, there seems to be no reason why streams of at least slightly higher ozone concentrations cannot be analyzed similarly.

PROCEDURE

A Gow-Mac four-filament thermal conductivity unit, Model RCT, with a 6-volt battery for power was employed. Through both arms of the cell dry oxygen was passed. The rate through each arm was not critical, but it was essential that it vary not more than about 10% throughout the preparation and subsequent use of the calibration curve. In these experiments, the oxygen flow through one arm was 1.0 liter per minute; through the other, also connected to the ozonizer, 0.6 liter per minute. The cell filament was adjusted to its operating value, 138 ma., and the oxygen flow started. After thermal equilibrium had been attained (about 1 hour), the unit was balanced by means of the zero balancing potentiometer on the cell control unit. The ozonizer, a Welsbach Model T-23 generator, was then started, its voltage adjusted, and the ozonized stream passed through one arm of the cell, also at a rate of 0.6 liter per minute. The ozone concentration of the stream reached a constant value in 3 to 5 minutes, at which time the cell voltage was measured to within ±0.05 mv. with a Rubicon potentiometer. Generally, 2 to 3 minutes were required for the conductivity cell to equilibrate. The ozone content of the gas was determined iodometrically (*1*).

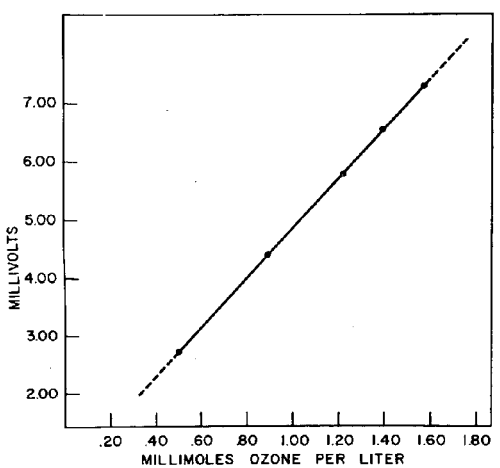

Figure 1. Relationship between ozone concentration and millivolts

The ozone concentration was then increased to the next desired value; the range investigated was 0.5 to 1.6 mmoles of ozone per liter of gas.

The cell retained its zero balance for the entire working day, and therefore the system did not have to be purged occasionally. At the end of the third day, however, the values obtained varied within 5% of the iodometric values, and the system was recalibrated. The voltage concentration data for the conditions employed in the laboratory are presented graphically in Figure 1.

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Purification of Di-2-naphthylthiocarbazone

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Di-2-naphthylthiocarbazone (2-naphthylazothionoformic acid) was originally synthesized by Suprunovich (*5*) and later in this laboratory by Hubbard and Scott (*4*). It has become an exceedingly useful compound for the quantitative determination of mercury in biological material (*1*, *3*, *6*). The compound used for this type of analysis must be very pure, showing a molecular extinction coefficient of approximately 42,000 at a wave length of 645 mμ, when chloroform is used as the solvent. In order to obtain this purity it is necessary to purify twice, as described by Hubbard and Scott (*4*). This purification process is tedious and the yield obtained for the final product is low.

Cooper and Kofron have published a sound and usable method of purification (*2*), but at present the authors are using a method which they feel is much more practical and very easy to manipulate. The refined compound obtained by the latter method meets the requirements for purity as given above.

Procedure. Place 0.5 gram of the di-2-naphthylthiocarbazone to be purified in a 100-ml. standard Griffin low-form beaker of Pyrex brand chemical glass No. 774 with pour-out. Dissolve in 30 ml. of reconditioned chloroform, mixing with a glass stirring rod to effect solution at a low temperature on the hot plate. Allow the chloroform solution to cool to room temperature and then transfer it to a round-bottomed, short ring-neck, Pyrex brand boiling flask, capacity 50 ml., equipped with a ⅌ joint No. 19/38. Place the flask directly on the ⅌ 19/38 joint of a rotating vacuum-type evaporator mounting at an angle of about 30° from horizontal. Grease the stainless steel ⅌ joint of the evaporator with a small amount of high vacuum silicone lubricant. Connect the side arm of the evaporator, which is a ⅌ 12/30 joint (for use with glass connections if desired), directly to a suitable vacuum supply. A water aspirator is satisfactory, as no trap is required. A three-way stopcock may be inserted in the vacuum line.

Turn on the vacuum and rotate the flask and contents, utilizing the built-in electric motor, for 30 minutes at 60 r.p.m. Release the vacuum and remove the flask. The remaining volume will be approximately 5 ml. Add 25 ml. of 200 proof ethyl alcohol, mix well by shaking, scrape the inside walls with a suitable glass stirring rod (slightly bent and flattened at the base) in order to remove particles that may adhere to the inside walls, and immediately immerse the flask and contents in a bath of acetone and dry ice for 10 minutes. Filter, using a Büchner porcelain funnel, and catch the precipitate on a No. 2 Whatman filter paper, 4.25 cm. in diameter. Rinse the flask with 10 ml. of 200 proof alcohol, again cool the flask and contents in the acetone–dry ice bath, and finally wash the precipitate with the alcohol. Remove the filter paper and contents to a watch glass, add to it any precipitate that adheres to the sides of the funnel, allow the compound to dry in the air, and weigh. The yield from 0.5 gram of the original compound should be approximately 0.3 gram.

Reconditioned Chloroform. Place 1 liter of chloroform, redistilled from a borosilicate glass still, in a 2-liter glass-stoppered borosilicate glass separatory funnel. Dissolve approximately 10 grams of hydroxylamine hydrochloride in 50 ml. of distilled water and make alkaline to phenol red indicator by the addition of reagent grade ammonium hydroxide. Add this solution to the chloroform and shake well. Allow the aqueous layer to separate and filter the chloroform through a fluted filter paper into a brown glass-stoppered bottle containing 20 ml. of absolute ethyl alcohol. Shake well and store in a refrigerator.

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