ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

International Microchemical Congress

IT IS our sincere hope that a large number of analytical chemists, and particularly those who are directly interested in microchemistry, will find it possible to attend the First International Microchemical Congress which will be held in Graz, Austria, July 2 to 6, 1950, under the auspices of the Austrian Society of Microchemistry (Österreichische Gesellschaft für Mikrochemie). The main purpose of this meeting is to provide an exchange of experiences in the microchemical field on an international basis. The Austrian Society of Microchemistry is to be commended for its initiative in sponsoring such a meeting.

A perusal of the preliminary program shows that outstanding lecturers, well known internationally in microchemistry, will cover all the more important fields, including fundamental research and applied methods and techniques. In addition, an effort will be made to coordinate the present activities in many countries looking towards the standardization of microchemical apparatus. Many of our readers are conscious of the distinct progress that has been in this direction in this country, largely through the work of the Committee on Standardization of Microchemical Apparatus, appointed by the Division of Analytical Chemistry of the AMERICAN CHEMICAL SOCIETY.

The occasion of the First International Microchemical Congress will serve to commemorate the important contributions in microchemistry made by F. Emich and F. Pregl. Emich died in Graz on January 22, 1940, at the age of nearly 80 years. He is frequently referred to as the father of microchemistry. Because of World War II, Emich has not received the international recognition for his many contributions that otherwise would have been accorded. It is most fitting that analytical chemists honor his memory at this time. A bust of Emich will be unveiled and placed in the now famous lecture hall where Emich inspired his students. A special lecture will be given on this occasion by A. A. Benedetti-Pichler, former student and co-worker of Emich. Originally the bust was to be made of terra cotta, but contributions from American microchemists have made it possible to provide a bronze bust of Austria's leading microchemist.

The Graz meeting will also serve to commemorate the 20th anniversary of the death of Pregl, who died on December 13, 1930, at the age of 61 years. Pregl's work received world-wide recognition. He was awarded the Nobel Prize in Chemistry in 1923 for his development of quantitative organic microanalysis. Hans Lieb, who, at present, is president of the Austrian Society of Microchemistry and president of the Committees for the International Congress, will lecture on the life and work of his co-worker.

In a world torn by misunderstandings and warring ideologies and facing difficult political, economic, and social complexities, international meetings of scientists and technologists take on new significance. Science is international, and meetings such as the one scheduled to be held in Graz this summer, the International Congress of Pure and Applied Chemistry, and 75th anniversary meeting of the AMERICAN CHEMICAL SOCIETY, scheduled to be held in the fall of 1951 in New York, provide an opportunity for scientists not only to exchange scientific information, but to build good will and understanding among the nations of the world. If the gatherings that have been planned during 1950 attract sufficient numbers, many other organizations concerned with science and technology will be encouraged to sponsor international meetings. Well-attended international meetings will counteract much of the present suspicion and misunderstanding that pervade the international political scene.

A New Service

BEGINNING with the January number ANALYTICAL CHEMISTRY has published each month a résumé of new products, including new equipment, apparatus, reagents, etc., and a digest of new industrial literature of special interest to the chemical analyst. The response from our readers to date has demonstrated the value of this service. Such information will be screened carefully by the editors, so that readers will find in these new departments only that which is really new and of value to them in their work.

Determination of Functionality in Organic Compounds

A symposium of six papers presented before the Division of Analytical and Micro Chemistry at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J., September 1949

Determination of Organic Functionality

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The scope and purpose of the symposium are outlined in an attempt to answer the questions: What type of method or methods should be used to solve a particular problem in organic analysis and what type of information can be obtained with what uncertainties? The relative nature of the problems confronting the analyst of inorganic substances and the analyst of organic substances is compared.

IN a certain sense, the present symposium is the result of the one organized by R. Bowling Barnes for the New York meeting of the AMERICAN CHEMICAL SOCIETY in September 1947. In this symposium (1), which covered the purity and identity of organic compounds, an attempt was made to deal with certain fundamental concepts pertaining to the purification and recognition of compounds. The present symposium seeks to define the basic factors which are involved in the quantitative estimation of organic functionality. The determination of organic functionality or organic functional behavior is a very necessary tool in the full realization of what was then defined as the three essential situations confronting the organic analyst. Barnes classified all of the problems of an organic nature which might be submitted to an analyst as being of three types: "(1) the determination of purity; (2) the establishment of identity; and (3) the determination of and proof of structure." Before further considering the relationship of the present discussion to the three types of problems mentioned, it might be well to define what is meant by functionality in organic compounds.

FUNCTIONALITY DEFINED

The term "organic functionality or organic functional grouping" should be considered in a broad sense as including not only such groups of atoms as carbonyl and carboxyl, but also such reactive groupings of atoms as unsaturation, enolizable carbonyl and active hydrogen, and such structural entities as terminal methyl groups—i.e., C-methyl groups. The term "determination of organic functionality" might well be extended in the apt phrase of Webb of Merck and Co. to include the "identification and quantitative determination of any structural moiety of an organic compound by methods other than direct isolation."

On the basis of the foregoing definition, one can readily see how the determination of functionality can be used in determining the purity, identity, and structure of organic compounds. Obviously, the determination of structure is predicated to a large extent on being able to estimate the type and amount of different structural entities in the molecule. Similarly, identity of a substance is best established by ascertaining what functional groupings of atoms are present. Finally, purity is often best established by measuring a functional grouping known to be present either in the matrix or in the impurity, but not in both.

FUNDAMENTAL APPROACH

In the present symposium an attempt is made to approach the problem of the determination of organic functionality from a more or less fundamental point of view. Specific methods are not discussed, except in so far as they illustrate or reinforce the general discussion of the technique involved. In each of the techniques which are considered the discussion will try to cover the following topics: (1) the basic principles involved in using the technique for measuring organic functionality; (2) the advantages and limitations as well as the pitfalls inherent in such a technique; (3) the general applicability of the technique in various situations and to various types of materials; and (4) the need for or probable direction of future development of the technique with, if predictable, an indication of possible fruitful lines of research.

In the discussion of specific techniques and problems, the assumption is made that the reader is, as a whole, conversant with the chemical reactions used, with the types of apparatus available, and with their mode of operation as well as with the general experimental procedure involved.

Fundamentally, the symposium tries to answer certain questions. What type of method or methods should be used to solve a given problem in organic analysis? What types of information will be obtained with the method? What will be the uncertainties in the information obtained? What are the general precision and accuracy obtainable? What reasons are there for preferring one method of attack to others for a given category of problems? What factors determine which technique will yield the best results for a given functional moiety in a given situation? What are the relative merits of the different techniques? Do the techniques give instantaneous information on the system under study? How can these techniques be used in conjunction with each other? What is the current status of these various techniques in reference to the problem of measuring organic functionality? What is the state of related techniques which are now but slightly used? An important further question considered is, of what value in organic analysis are the accessory measurements such as ionization constants, cracking patterns, and similar physical characteristics which are obtainable by the analytical measurement?

SCOPE OF SYMPOSIUM

The scope of the symposium is much broader than can be covered in the time available. However, it is this greater breadth of approach which is desirable and which is actually being used in the more fruitful approaches to the complex problem of the determination of organic functionality.

It is obvious that it will not be possible to include all the possible techniques that may be used. For example, practically no mention is made of the use of isotopic tracer techniques as an aid in the determination of organic functionality. Analogously, certain other important techniques such as mass spectrometry and fluorescent phenomena are given, perhaps, less attention than their present or future importance would justify. In preparing the program, those topics were selected which would be of most interest as reflecting the present status of the field as well as future development. Here, of course, one is confronted by the dilemma of the relative amount of attention to be given the more widely used techniques which are by that very fact better known, as compared to the less widely used techniques which are less well known. There is the factor involved of how much emphasis should be placed on potentially useful techniques as compared to those of known value. The authors within the severe time limitations imposed on them have attempted to strike a fair balance.

The papers included in the symposium are of two types. Three papers survey the three broad groups of techniques that are at present being used in the determination of organic functional groups: chemical reactivity, molecular spectroscopy, and electrical measurements. The second group of two papers consists of case histories which make concrete the previous discussion of techniques. These two papers are devoted to the illustration of how the techniques described as well as other techniques have been applied to research and control on two important classes of materials which are typical of the problems involving the analyst of organic substances. The two case histories survey the analysis of cellulose derivatives and the methods used in the functional group analysis of the penicillins. These case histories should serve to emphasize how the different kinds of information obtainable by different methods and techniques complement, supplement, and amplify each other.

In considering the problem which confronts the analyst of organic substances, it might be pertinent to consider the nature of his problem in reference to that of the analyst of inorganic substances. Lundell and Hoffman (*3*) have indicated the frequency with which the various elements of the periodic table are determined. Although their table was published eleven or twelve years ago, the relative frequency with which the elements are determined has not altered to a great extent if one excepts the determination of those elements essential in the exploitation of atomic energy. Of this great variety of elements which are determined by the inorganic analyst or, rather, by the analyst of inorganic materials, only very few are determined by the organic analyst. Very few organic analysts ever have occasion to determine elements other than carbon, hydrogen, nitrogen, sulfur, and the halogens. Phosphorus is occasionally determined and the importance of the determination of oxygen in organic compounds is increasing. The difficulty that the organic analyst encounters in determining his few elements is due to the fact that his elements are differently bound as compared to those of the inorganic analyst. The inorganic analyst is solely concerned with the determination of ions. The organic analyst, on the other hand, often has as a preliminary step the conversion of his elements either into ions or into inorganic simple molecules which in the form of a gas can be suitably absorbed or otherwise measured.

The situation in reference to the determination of organic functional groups is somewhat analogous. The organic functional groups that are commonly determined in laboratories where general organic analytical work is performed include active hydrogen, acyl, alkimide and alkoxyl, amine, primary amine, amino acid, carbonyl, carboxyl, C-methyl, hydroxyl, and unsaturation. The number is relatively small and is in many cases limited by the ability of the analytical chemist to devise organic reactions which have yields in excess of 95%. The difficulty of stoichiometric or complete organic reactions is so well accepted as an almost fundamental postulate of synthetic organic chemistry that it is highly complimentary to the analytical chemist that his ability to obtain yields in the neighborhood of 98 to 100% is accepted without undue comment. However, the number of functional entities which the analytical chemist is now called upon to measure has increased tremendously, as can be seen in the recent compilation by Barnes and co-workers (*2*) of some of the functional groups which are commonly determined by infrared absorption spectrophotometry. The use of both new and old chemical reactions, and the further utilization of techniques such as polarography, Raman spectroscopy, mass spectrometry, and x-ray diffraction are still further extending the limits which encompass the kinds of functional groups as well as the number of different functional groups which can be analytically determined.

ULTIMATE GOAL

The ultimate goal in the determination of organic functional groups is simply the determination of compounds as such—that is, not the reporting of the percentage of hydroxyl group content in terms of an alcohol presumed to be present but the actual measurement of the alcohol by methods which would serve to distinguish it from its congeners as well as other related compounds. X-ray diffraction, petrographic microscopy, and some types of molecular spectroscopy are now making this possible. It is hoped that in the near future it will be possible to hold an offspring symposium, covering not the determination of organic functionality but the determination of organic compounds.

LITERATURE CITED

(1) Barnes, R. B., *et al.*, ANAL. CHEM., 20, 96 (1948).
(2) Barnes, R. B., Gore, R. C., Stafford, R. W., and Williams, V. Z., *Ibid.*, 20, 402 (1948).
(3) Lundell, G. E. F., and Hoffman, J. I., "Outlines of Methods of Chemical Analysis," p. 13, New York, John Wiley & Sons, 1938.

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Determination of Organic Functional Groups by Chemical Means

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In order to use a chemical reaction for determining a functional group, at least one of the reactants or products must be measurable. Reactions that liberate or consume an acid, base, oxidant, reductant, or gas are particularly adaptable for quantitative analytical purposes. Several reactions which liberate or consume easily determinable compounds such as water can be used to measure functional groups. Reactions that yield precipitates also have analytical possibilities. Equilibrium, incomplete reaction, poor end points, and interferences are the troubles usually encountered. Techniques are available for minimizing these difficulties.

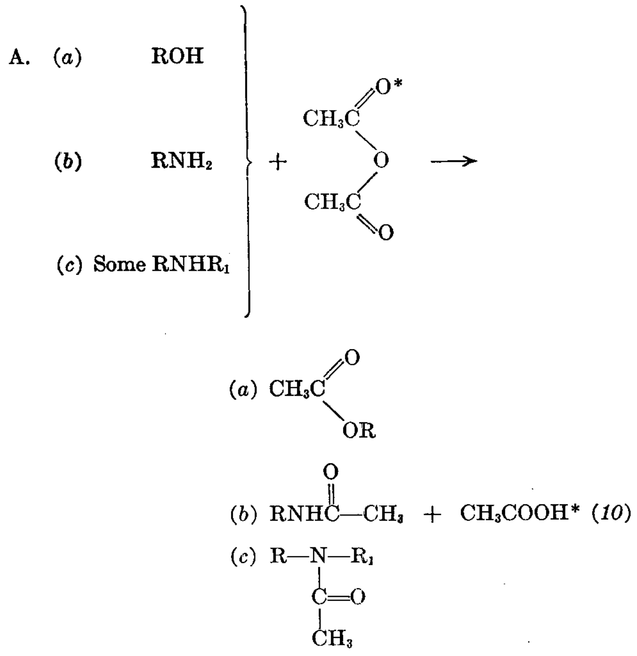
THE methods available for functional group determination have been amply described and reviewed (*15, 22*). Methods exist for practically all the functional groups, but situations often arise when changes in methods, or sometimes a completely new procedure, are necessary. This paper is concerned with the techniques and reasoning involved in devising a new method of analysis or making changes in known procedures.

CHEMISTRY OF FUNCTIONAL GROUPS

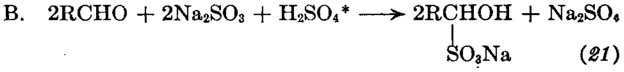
When a new procedure is necessary, the chemistry of the functional group in question must be completely surveyed. The factors involved are: the presence of at least one reactant or product that can be measured, the completeness of the reaction, and effect of impurities.

A reaction in which a base, acid, oxidant, or reductant is liberated or consumed is a good one to use for functional group determination. These materials are very easily measured by common analytical means. In the following examples of known analytical methods which use reactions of this type, the asterisk indicates the component determined.

Acid Consumed



B.



$$2RCHO + 2Na_2SO_3 + H_2SO_4^* \longrightarrow 2R\underset{\displaystyle SO_3Na}{CHOH} + Na_2SO_4 \qquad (21)$$

$$\left.\begin{array}{l}(a)\ RCH(OR_1)_2\\ (b)\ ROCH{=}CH_2\end{array}\right\} + H_2O \xrightarrow{H^+}$$

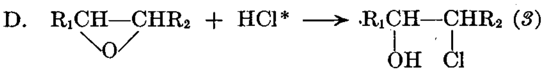
(*a*) RCHO + (*a*) $2R_1OH$

(Aldehydes determined
as in above equation)

(*b*) CH_3CHO + (*b*) ROH (*14*)

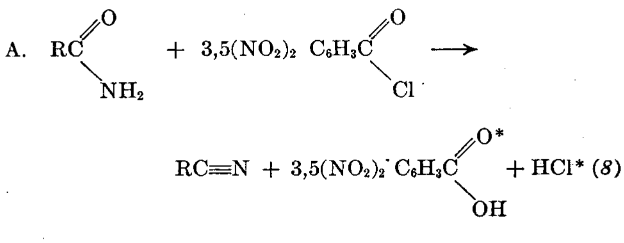
C. Titration of basic materials such as amines (primary, secondary, tertiary), pyridine, quinoline, carboxylic acid salts, etc., with standard acid solutions. (Where water gives unsatisfactory results because of insolubility or poor end point, a mixture of 1 to 1 ethylene glycol and isopropyl alcohol can be used, *11*).

D.

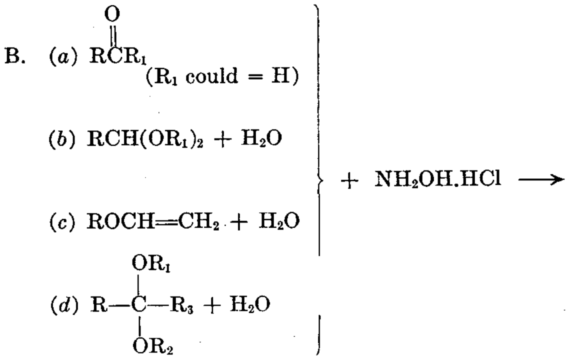


Acid Produced

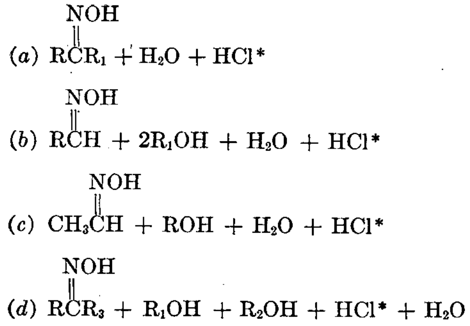
A.



B.



$$\left.\begin{array}{l}(a)\ R\overset{O}{\overset{\|}{C}}R_1\ (R_1 \text{ could } = H)\\ (b)\ RCH(OR_1)_2 + H_2O\\ (c)\ ROCH{=}CH_2 + H_2O\\ (d)\ R-\underset{OR_2}{\overset{OR_1}{C}}-R_3 + H_2O\end{array}\right\} + NH_2OH.HCl \longrightarrow$$



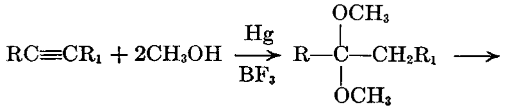
$$(a)\ \mathrm{R\overset{NOH}{\overset{\|}{C}}R_1 + H_2O + HCl^*}$$

$$(b)\ \mathrm{R\overset{NOH}{\overset{\|}{C}}H + 2R_1OH + H_2O + HCl^*}$$

$$(c)\ \mathrm{CH_3\overset{NOH}{\overset{\|}{C}}H + ROH + H_2O + HCl^*}$$

$$(d)\ \mathrm{R\overset{NOH}{\overset{\|}{C}}R_3 + R_1OH + R_2OH + HCl^* + H_2O}$$

C. $\mathrm{RC{\equiv}CH + 2AgNO_3 \longrightarrow RC{\equiv}CAg.AgNO_3 + HNO_3^*}$ (*1*)

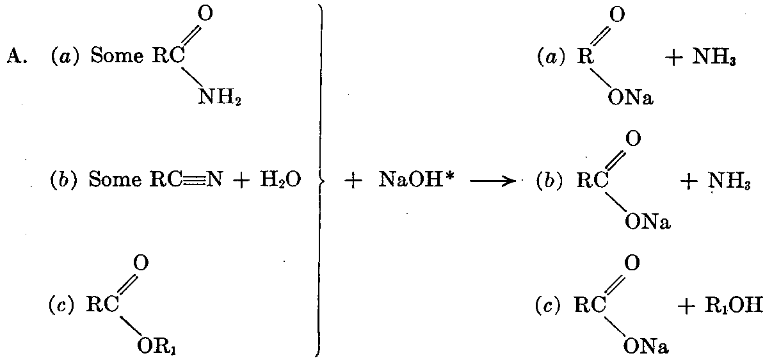


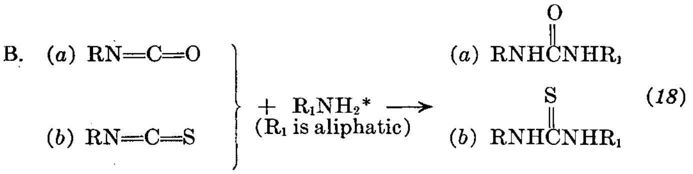
$$\mathrm{RC{\equiv}CR_1 + 2CH_3OH \xrightarrow[BF_3]{Hg} R{-}\underset{\underset{OCH_3}{|}}{\overset{\overset{OCH_3}{|}}{C}}{-}CH_2R_1 \longrightarrow}$$

ketal determined as in B (*23*)

D. Dehydrohalogenation (applicable only to some halogen compounds; a general equation cannot be written).

Base Consumed



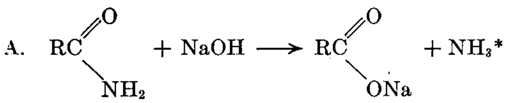


$$\left.\begin{array}{ll}\text{B. (a)} & \mathrm{RN{=}C{=}O}\\ \text{(b)} & \mathrm{RN{=}C{=}S}\end{array}\right\} + \mathrm{R_1NH_2^*} \longrightarrow \begin{array}{ll}\text{(a)} & \mathrm{RNH\overset{O}{\overset{\|}{C}}NHR_1}\\ \text{(b)} & \mathrm{RNH\overset{S}{\overset{\|}{C}}NHR_1}\end{array} \quad (18)$$

($\mathrm{R_1}$ is aliphatic)

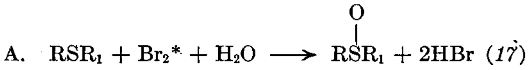
C. Titration of acids with standard alkali.

Base Produced



B. $\mathrm{RCOOX \xrightarrow{Ignition} X_2CO_3}$ (*20*)
(X = Na, K, Ca, or Ba with proper valences taken into account)

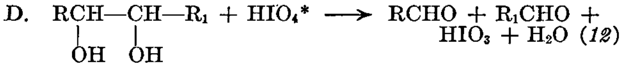
Oxidant Consumed



A. $\mathrm{RSR_1 + Br_2^* + H_2O \longrightarrow R\overset{O}{\overset{|}{S}}R_1 + 2HBr}$ (*17*)

B. $\mathrm{RSSR_1 + 5Br_2^* + 4H_2O \longrightarrow RSO_2Br + R_1SO_2Br + 8HBr}$ (*17*)

C. $\mathrm{2RSH + I_2^* \longrightarrow RSSR + 2HI}$ (*4*)



D. $\mathrm{R\underset{OH}{\underset{|}{C}}H{-}\underset{OH}{\underset{|}{C}}H{-}R_1 + HIO_4^* \longrightarrow RCHO + R_1CHO + HIO_3 + H_2O}$ (*12*)

Oxidant Produced. Iodine liberated from iodides by peroxides is measured (not applicable to all organic peroxides) (*5*).

Reductant Consumed

A. $\mathrm{TiCl_3}$ reductions of —$\mathrm{NO_2}$, —NHNH—, —N=N—, and diazonium salts ($\mathrm{TiCl_3^*}$ is measured) (*2, 15*).

B. $\mathrm{As_2O_3}$ consumed on reaction with peroxides is measured (*13*).

Reactions in which a gas is liberated or consumed can also be used for quantitative determination of functional groups. A liberated gas can be collected and measured—i.e., the nitrogen liberated on decomposition in the determination of diazonium compounds and hydrazines.

$$\mathrm{RN{\equiv}NCl + H_2O \xrightarrow{\Delta} ROH + N_2 + HCl}$$

$$\mathrm{RNHNH_2 \xrightarrow{Cu^{++}} RN{\equiv}NCl + H_2O \longrightarrow ROH + N_2 + HCl}\ (19)$$

Active hydrogen determination by the Grignard reagent, in which methane is liberated, is another example. In quantitative hydrogenation the quantity of gas consumed is used as a measure of a functional group.

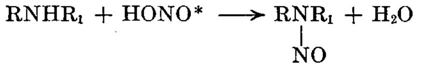
Reactions in which such easily determinable materials as water, silver ions, and sodium nitrite are used or produced are also applicable to determining functional groups. Water takes part in, or is formed in, the reaction of many functional groups; a system of analysis has sprung up around this basis of measurement and is described by Mitchell and Smith (*9*). Silver enters into reactions with mercaptans (thiols) and acetylenic hydrogen compounds and is easily determined by standard methods.

$$\mathrm{RSH + AgNO_3^* \longrightarrow RSAg + HNO_3}\ (6)$$

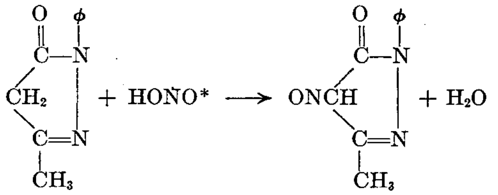
$$\mathrm{RC{\equiv}CH + 2AgNO_3^* \longrightarrow RC{\equiv}CAg.AgNO_3 + HNO_3}\ (1, 15)$$

Sodium nitrite takes part in many organic reactions as nitrous acid and can be used to determine primary aromatic amines by diazotization and some secondary aromatic amines and active methylene group compounds by nitrosation.

$$\mathrm{RNH_2.HCl + HONO^* \longrightarrow RN{\equiv}NCl + 2H_2O}$$



$$\mathrm{RNHR_1 + HONO^* \longrightarrow R\underset{NO}{\underset{|}{N}}R_1 + H_2O}$$



TIME OF REACTION

A reaction used for a quantitative measurement has to go to completion in a reasonable length of time; the shorter the time, the better. The time necessary for complete reaction can be shortened by several means.

A. Increasing concentration of reagents used to react with the functional group, especially if one of the products of the reaction is being determined. If the excess of reagent is being determined, it will be necessary to determine too large an excess, and the analysis will be based on the difference between two large figures, which is never desirable.

B. Increasing the temperature of the reaction by using higher boiling solvents than are ordinarily used, so that reflux temperatures will be higher.

C. Use of a more reactive reagent.
D. Resorting to higher pressure reactions in some instances.
E. Catalysts.

Technique A is employed in the acetylation of hydroxyl and amino groups, mentioned above. At least 1 part of acetic anhydride to 3 parts of pyridine must be used to operate in a reasonable length of time. If more pyridine is used, the reaction time increases sharply. In the dehydrochlorination of 1-chloro-2-methoxy-2-phenylethane, if 0.5 *N* sodium hydroxide is used, the reaction proceeds to only 12% of completion in 4 hours; if 5 *N* sodium hydroxide is used, the reaction is complete in 1.5 hours.

The saponification of some esters requires the use of technique B. Alcoholic (methanol) sodium hydroxide is usually used for saponification of esters. However, some esters do not saponify readily, and the reflux temperature of the methanol is too low to permit complete reaction in a reasonable time. The use of a higher alcohol overcomes this difficulty. Ethylene glycol is often used as the solvent, though it is seldom heated to its boiling point because of its decomposition at that temperature.

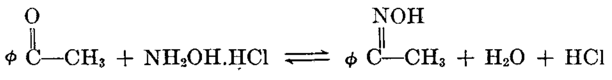
The dehydrochlorination of methyl-α-chloroacrylate can also be used to exemplify the application of technique B. If ethyl alcohol is used as a solvent, the dehydrochlorination proceeds to about 70% completion in 2 hours; with trimethylbenzene (mixture of various isomers, boiling point 170° C.), the reaction goes to completion in 0.5 hour.

The dehydrochlorination of methyl-α-chloroacrylate can also be used to demonstrate technique C. If pyridine is used as the dehydrochlorinating agent, no reaction takes place; with piperidine, which is a much stronger base, the reaction proceeds to completion in 0.5 hour. When applied to the dehydrochlorination of 1-chloro-2-methoxyl-2-phenylethane, pyridine yields no reaction in 2 hours, piperidine yields about 75% reaction, and 5 *N* sodium hydroxide (a stronger base than piperidine) yields complete reaction.

Technique D is applicable only when one of the reactants is a gas. In quantitative hydrogenation, the reaction proceeds at a faster rate the higher the pressure. In hydrogenation of vinyl alkyl ethers, a 20-gram sample is saturated in about 2 hours at 10 cm. of mercury, or in about 0.5 hour at 50 cm.

Catalysts (technique E) can sometimes be employed to speed up a reaction. The use of mercuric sulfate will speed up the addition of bromine to an unsaturated linkage (*7*). 2-Heptyne will brominate completely in 7 minutes in the presence of mercuric sulfate, whereas without the catalyst the results are 23% low under the same conditions. Dichloroethylene will brominate completely in 5 minutes with the catalyst, but without the catalyst it will go only 2% toward completion in 20 minutes.

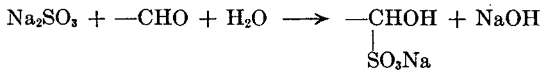
The completeness of a reaction is often affected by the existence of an equilibrium. There are several ways of eliminating or at least minimizing the effects of an equilibrium. The reaction can be pushed closer to completion by using a higher concentration of reagent, or can be pulled to completion by removing one of the products from the reaction. The latter method is preferable. The reaction of acetophenone and hydroxylamine hydrochloride is a good example of how equilibrium difficulties can be overcome.



$$\phi\,\overset{O}{\overset{\|}{C}}-CH_3 + NH_2OH.HCl \rightleftharpoons \phi\,\overset{NOH}{\overset{\|}{C}}-CH_3 + H_2O + HCl$$

In an aqueous system, the reaction equilibrium does not permit the reaction to proceed more than about 50% to completion. In a nonaqueous solvent (1 to 1 ethylene glycol–isopropyl alcohol), the reaction goes to 80% completion. When a weak base, di(β-hydroxyethyl)aniline, is added to the solvent mixture to tie up the hydrogen chloride liberated, the reaction proceeds to 100% completion. If water is added to the system containing even the weak base, the equilibrium is set up again.

Equilibrium difficulties are also encountered in determining carbonyl compounds using sodium sulfite.



$$Na_2SO_3 + -CHO + H_2O \longrightarrow \underset{SO_3Na}{\underset{|}{-CHOH}} + NaOH$$

When a carbonyl compound is introduced into a solution of sodium sulfite, the pH of the solution rises, indicating liberation of the hydroxide. On titration of the hydroxide, a few milliliters of acid bring the pH of the solution back to the original pH of the sulfite, but on standing the pH rises again as more hydroxide is formed. On addition of an excess of standard acid, the reaction can be brought to completion. Titration of the excess of acid indicates the amount of carbonyl compound. However, as the excess acid is consumed and the end point is approached, the bisulfite addition compound breaks up and equilibrium is again reached. This action tends to dull the end point. To minimize this effect, a large excess of sodium sulfite is used to keep the reaction essentially at completion as the excess of acid is consumed. When aliphatic aldehydes through the butyraldehyde are determined, on addition of the sample to the sulfite, the hydroxide liberated can be titrated if time is allowed between additions of acid for the reaction to proceed further. The problem here is the low boiling points of some of these aldehydes, which cause loss. On addition of excess acid to the sulfite solution, the reaction is so near completion that no aldehyde odor can be noted, in spite of the low boiling points and strong odors of aldehydes. On back-titration of the excess acid, as soon as the end point is close, the odor of free aldehyde becomes noticeable, showing the presence of an equilibrium.

When aldehydes such as benzaldehydes are determined, the equilibrium is noticeable, and the end point on back-titration of the excess acid is dulled to a significant extent, but results can still be obtained.

With acetone and most ketones, even a large excess of sulfite cannot keep the reaction anywhere near completion. As the acid is consumed on the back-titration, the bisulfite addition compound breaks down and the reaction proceeds in the reverse direction. As a result, no end point is visible.

END POINTS

The problem of poor end points often plagues the analyst. It is of special significance in the determination of functional groups, because most of the work is done in nonaqueous media, and, if indicators show a poor end point, a pH meter cannot usually be used. For instance, if a saponification is called for on a highly colored sample, the color of the sample makes it impossible to see any indicator change, and a pH meter has to be used to ascertain the end point. If the sample is water-insoluble, alcoholic sodium hydroxide must be used for the saponification, and the electrodes used with a pH meter will not operate in such a medium. However, if after the saponification an amount of water is added greater than the amount of alcohol present, the ordinary glass and calomel electrodes can be used with a pH meter to give a potentiometric indication of the end point. This method for getting a pH meter to work in an adverse system can be applied only when the component being titrated will not be forced out of solution by the water.

A method for titrating potentiometrically in a nonaqueous medium (*11*) uses propylene or ethylene glycol and a hydrocarbon solvent (generally isopropyl alcohol). A titration curve can be obtained in this solvent mixture, but the absolute pH readings have no meaning. A definite break is produced on plotting milliliters of reagent against "apparent pH."

The intensity of the breaks in the titration curves in the glycol-alcohol mixture is much greater than for the same samples in a water system. Weak bases such as aniline, mono- and diethylaniline, pyridine, quinoline, and carboxylic acid salts all yield substantial breaks in apparent pH when titrated with acids, but in water solution the curve at best is very poor.

This glycol solvent mixture is a good solvent for many different compounds, so that the entire reaction and final titration can be carried out in the same medium. In the determination of acetophenone with hydroxylamine hydrochloride, discussed above, water had to be avoided for reasons of equilibrium, and the indicator end point was poor. When ethylene glycol–isopropyl alcohol (1 to 1) was used as solvent, the final mixture could be titrated with a pH meter.

INTERFERENCES

In functional group analysis, as in all types of analysis, the problem of interferences arises. A sample which contains a component that interferes with the desired analysis can be treated in several ways.

A. By physical means (distillation, adsorption, extraction) to separate the desired component from the interference.
B. Chemically, to alter the interference into a compound that is harmless to the analysis.
C. By a method that determines both the desired component and the interference; then the interference alone is determined and the desired component is obtained by difference.

An example of technique A is the determination of free phenol in a phenolic resin by bromination. If the bromination procedure is carried out on the sample as is, the analysis for the phenol will be high, owing to substitution of bromine on the resin. If the phenol is separated from the resin by dissolving the sample in chloroform and extracting the free phenol from the solution using aqueous sodium hydroxide solution, the aqueous extract may be used for the determination.

An example of technique B is the determination of diethylaniline in the presence of monoethylaniline and aniline. All three materials are very basic in glycol–isopropyl alcohol and can be titrated. On reaction of the sample with acetic anhydride, the aniline and monoethylaniline are converted to the corresponding anilides, which are not basic; then the reaction mixture can be titrated directly with standard acid for diethylaniline in the glycol–isopropyl alcohol medium.

In the same system of anilines, ethyl alcohol is also present (aniline reacts with ethyl alcohol to produce the substituted anilines). To determine the ethyl alcohol, the sample is quantitatively acetylated; ethyl alcohol, aniline, and monoethylaniline are acetylated. The excess acetic acid and anhydride are titrated to the neutral point with sodium hydroxide solution, and an excess of standard sodium hydroxide is added. Only the ester is saponified; the anilides are not affected.

Technique C can be demonstrated in the analysis of mixtures of acetals and vinyl ethers. The total ether plus acetal can be determined by hydrolyzing the sample and determining the acetaldehyde formed (*14*). On a separate sample, the vinyl ether can be determined iodometrically (*16*). The acetal is obtained by difference.

Another example of technique C is the determination of the components of a mixture of $HOCH_2C{\equiv}CCH_2OH$, $HOCH_2CH{=}CHCH_2OH$, and $HOCH_2CH_2CH_2CH_2OH$. A quantitative acetylation picks up all three components. A quantitative bromination picks up the unsaturated components. The acetylenic member of the series is picked by the method of Wagner, Goldstein, and Peters (*23*). By subtracting the analysis for the acetylenic compound from the bromination analysis, the ethylenic compound can be obtained. By subtracting the bromination analysis from the acetylation, the saturated butanediol is obtained.

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Determination of Organic Functionality by Molecular Spectroscopy

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IN RECENT years analytical chemistry has experienced, with great impact, the inclusion of methods and techniques that utilize molecular spectroscopy. Certain classical analyses and tests have been essentially replaced with an increase in efficiency, accuracy, and information obtainable. Inherent and basic in this process is the fact that by the techniques of molecular spectroscopy information of a very fundamental and characteristic nature is obtained. Because of these successes, there has arisen a rather widespread notion that such techniques will with time become omnipotent with regard to analytical problems. This is not the case, and hence it is important, at this time, to discuss the limitations as well as the scope and generality of the methods now available and those that may be reasonably anticipated. Many of those who are not directly engaged in absorption spectroscopy believe that infrared, Raman, ultraviolet, and mass spectroscopy are competitive in the sense that the same problems may be solved with their use. Although this is largely true for the first two, the latter two furnish information of a completely different nature and there is relatively little overlapping of application.

INFRARED AND RAMAN SPECTROSCOPIC METHODS

A molecule is not a rigid structure. It comprises an assembly of atoms that are constrained by their mutual interactions to define equilibrium positions. The valence bonds are elastic in nature and hence the geometrical structure that is the molecule can vibrate and rotate in a manner analogous to bodies of macroscopic experience. Excepting collisions between molecules, the principal means whereby the vibrations of this geometrical structure may absorb or give up energy is by the absorption or emission of radiant energy.

A molecule will possess definite modes of vibration and hence will show strong absorption only to those wave lengths that will excite these modes. In the classical theory the molecule is regarded as absorbing electromagnetic frequencies that are identical with its own vibrational frequencies. From quantum mechanics we know that this is not the most correct interpretation. However, for the analytical applications and for work in determination of molecular structure this interpretation is adequate.

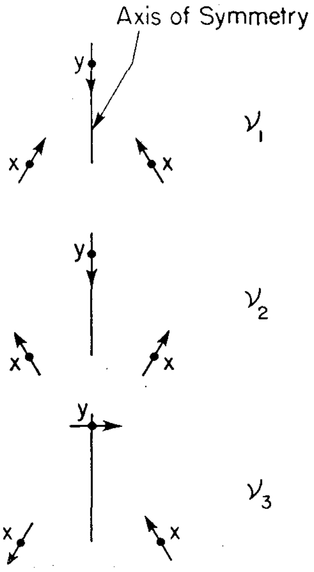


Figure 1. Vibrational Modes for Nonlinear YX_2 Molecule

In the simplest type of molecule, the diatomic, the frequency of the vibration wherein the two atoms move back and forth toward each other along the valence bond is given by:

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \tag{1}$$

where k is the force constant for the valence bond and μ is the reduced mass, being given by $\mu = m_1m_2/(m_1 + m_2)$ where m_1 and m_2 are the masses of the atoms involved. It should be noted from Equation 1 that as the valence bond strength increases, k will increase, with an increase in the vibrational frequency. As the masses of the constituent atoms increase, it will result in a decrease of the frequency.

As the complexity of the molecule increases, so also do the number and complexity of the possible vibrational modes. In Figure 1 may be seen the three modes for the triangular triatomic molecule, YX_2. In principle, it is possible to calculate the frequencies and determine the modes for any molecule. Except for the simpler ones, this meets with the most formidable mathematical difficulties and such results have been obtained on only a very few of the total number of known molecular species. The practical application of vibrational spectroscopy to the determination of structure of complex molecules must therefore be pursued by essentially empirical methods. These utilize the results extrapolated from the study of simpler molecules as well as correlations obtained by examination of series of homologous and similar compounds.

In infrared absorption spectroscopy, the fraction of light energy absorbed by the sample as a function of wave length is determined. Such data show regions or bands of intense absorption, each of which may be correlated with a particular vibrational mode. In general, the bands may be regarded as falling into either of two classes, bands characteristic of group-type (such as O—H, C=O, N—H, C—C, etc.) vibrations and bands due to over-all or skeletal vibrations. The former vibrational frequencies are essentially constant, independent of the remainder of the molecule, and hence furnish a most valuable tool for determination of molecular structure. As the latter depend specifically upon the geometry, constituent atoms, and valence forces of the molecule, they furnish a unique pattern or "fingerprint" for each molecule species. This allows the use of the spectra for identification (through comparison with spectra of known materials) of unknown materials and furnishes the basis for the analytical applications of spectra wherein unique bands are utilized for quantitative determinations.

In Raman spectroscopy the sample is irradiated with monochromatic ultraviolet or visible light. When the light scattered at right angles by the sample is spectrally examined, it is found that it is not all of the same frequency as the incident light. Some of the radiation is displaced in small but definite amounts from the frequency of the incident light. These displacements are called Raman shifts. Through a process of absorption and re-emission of the incident light, energy is either added to or subtracted from the molecular vibrational mode, just as an infrared absorption band is found for each infrared active vibrational mode. Hence the same fundamental information is furnished by the two techniques. There are some exceptions to this, based on symmetry elements in the molecules, but for the requirements of analytical chemistry they are relatively minor.

The experimental details of instrumentation and operation in infrared and Raman spectroscopy have been abundantly published (*3*, *57*). At the present time infrared absorption is much more intensively applied than are Raman spectra. Infrared equipment has been more generally available and the techniques are simpler. The material in this paper is discussed largely in terms of infrared absorption. In a general way, the scope and limitations that apply to infrared absorption also apply to the Raman methods.

When the capacity of each molecule in a sample to absorb radiation is independent of its environment, Beer's law of absorption applies. This is given by:

$$D = \log (I_0/I) = acl \tag{2}$$

where D is known as the optical density, I_0 and I are the intensities of the incident and transmitted energies, respectively, a is a constant depending upon the material and upon the wave length, c is the concentration of the material, and l is the thickness of the sample. If a constant absorption cell thickness is used, it is common practice to denote product al as the calibration coefficient, b. If several materials are present—three, for example—Beer's law becomes:

$$D_1 = b_{11}C_1 + b_{12}C_2 + b_{13}C_3 \tag{3}$$

where D_1 refers to a particular wave length λ_1, b_{11} is the calibration coefficient for the first compound at that wave length, b_{12} and b_{13} apply similarly to the second and third compounds at the same wave length, and C_1, C_2, and C_3 refer to the concentrations of the first, second, and third compounds, respectively. If such data are taken at three separate wave lengths, three simple linear equations are obtained which may be solved for the concentrations, thus yielding the analysis.

For a successful analysis of this type, two conditions must in general be fulfilled. In order to obtain the calibration coefficients, it is necessary that pure compounds be available. In order to solve the sets of linear equations with satisfactory accuracy, it is important that optical discrimination be achieved. By optical discrimination is meant that at each wave length used, one compound absorbs more strongly than the others and that each compound is represented by one such wave length.

Gas Analysis. The analysis of C_4 hydrocarbon mixtures containing *n*-butane, isobutane, 1-butene, isobutylene, *cis*-2-butene, *trans*-2-butene, and 1,3-butadiene has probably been the most outstanding application of infrared absorption to gas analysis (*8*). Figure 2 shows the absorption spectra of three of the gases mentioned. This demonstrates how the data may be plotted in such a way as to simplify the choice of wave lengths to

In recent years methods and techniques that utilize molecular spectroscopy have replaced many classical analyses and tests with an increase in efficiency and accuracy. Information of a very fundamental and characteristic nature is obtained. Although infrared and Raman spectroscopic methods may often be used to solve the same problems, ultraviolet and mass spectroscopy furnish information of a completely different nature and there is relatively little overlapping of application. The limitations as well as the scope and applicability of molecular spectroscopy methods are discussed.

use in the analysis. At each wave length designated by an arrow in the figure, one compound always absorbs much more strongly than the others.

Suitable wave lengths may be found for all the above C_4's and it is possible to set up the equations and organize a very efficient and effective analysis procedure. The accuracy is very good, the average errors being of the order of 0.5% total sample or less. A typical analysis on a synthetically blended sample may be seen in Table I. These data were taken under completely routine conditions and represent the accuracy attainable in day-to-day operations. It is important that the sample be fairly free of C_3 and C_5 components, as a few per cent of either can cause serious errors. This is usually achieved by making a C_4 cut on low temperature distillation equipment.

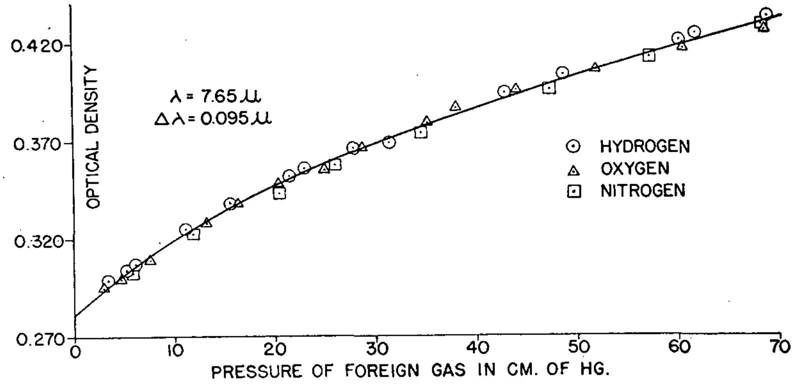


Figure 3. Optical Density of Constant Pressure of Methane Plotted against Pressure of Foreign Gas (*20*)

Methane, acetylene, carbon monoxide, sulfur dioxide, and other gases of low molecular weight cannot be analyzed by this method, owing to an effect known as pressure broadening. Because of this effect, the absorption due to any one of these gases depends not only on its concentration, but also upon the total pressure in the cell, and Beer's law is not obeyed. Methods suitable for such systems are discussed below. Other gaseous hydrocarbons in the C_3 and C_5 range may readily be analyzed in the gas phase by the above method. An interesting application of the use of this technique for the control of plant operations by the analysis of multicomponent C_5 mixtures has recently been described (*60*).

Symmetrical diatomic gases such as oxygen, hydrogen, and nitrogen, and the monatomic gases possess no absorption in the infrared. However, except that the gases must obey Beer's law, there are no particular restrictions on what mixtures of gases will yield to the above procedure. Raman spectroscopy is not very well suited to gas analysis because of the low scattering power of the gas, which is a result of the relatively low molecular density.

Table I. Typical Infrared Analysis of Synthetic Blend of C_4 Hydrocarbons (*15*)

Compound	Synthetic, %	Calculated, %	Difference, %
n-Butane	19.7	19.1	−0.6
Isobutane	10.4	10.6	+0.2
1-Butene	19.8	20.4	+0.6
Isobutylene	16.8	16.9	+0.1
cis-2-Butene	14.8	14.6	−0.2
trans-2-Butene	18.5	18.3	−0.2
Butadiene	0.0	0.1	+0.1

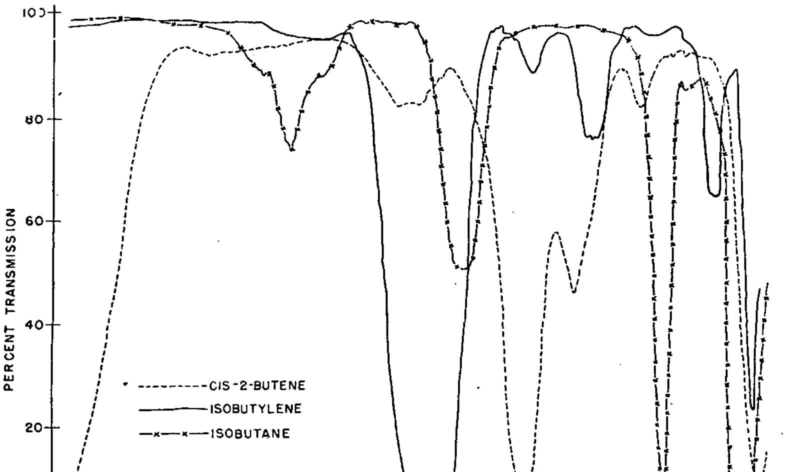


Figure 2. Infrared Absorption Spectra of Three C_4 Hydrocarbon Gases between 7 and 14μ (*15*)

Trace analysis may sometimes be done by the above procedure. However, for high sensitivity in trace analysis, the gas that one is seeking must possess a very much larger absorption coefficient at a particular wave length than any of the major constituents; otherwise the absorption of the latter will obscure the absorption of the trace gas at low concentrations. If suitable wave lengths can be found, it is sometimes possible to increase sensitivity by the use of very long path lengths or of high pressure gas cells; concentrations of water as low as 1 p.p.m. may be determined in a number of commercial refrigerants (*7*).

Significant future developments in infrared gas analysis will probably be in improved apparatus and technique, allowing greater accuracy of optical density determinations in shorter times.

Analysis of Gases Not Obeying Beer's Law. The gases of low molecular weight do not obey Beer's law and manifest the phenomenon of pressure broadening. Their absorption depends not only upon the molecular density but also on the total pressure. This is a consequence of broadening of the individual rotational lines as a function of the time between collisions (*63*).

Data illustrating the pressure-broadening effect may be seen in Figure 3, where the optical density of a constant partial pressure of methane is plotted as a function of the pressure of nonabsorbing or foreign gas present. If Beer's law were obeyed by the methane, all pressures of foreign gas would result in the same optical density value. It is merely fortuitous that the effects of hydrogen, oxygen, and nitrogen are essentially identical for the case demonstrated. In general, it has been shown that different foreign gases will have different effects and that the order of effects cannot always be extrapolated from one absorption band of the absorbing gas to another (*21*).

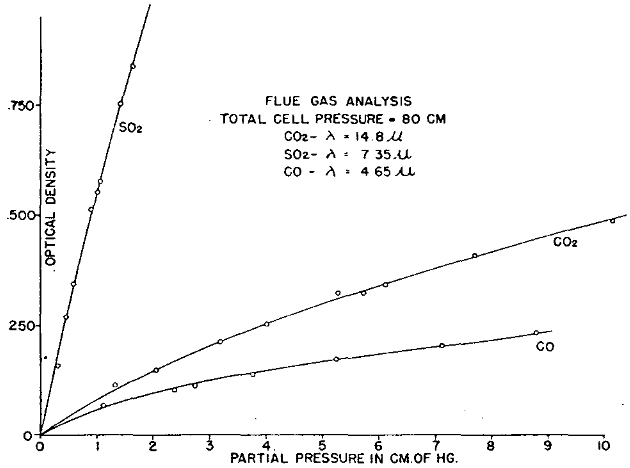


Figure 4. Calibration Curve for Determination of Carbon Dioxide, Sulfur Dioxide, and Carbon Monoxide in Gaseous Mixtures (*20*)

Constant cell pressure maintained for all samples

If the pressure-broadening effects of the foreign gases are known, it is possible in some cases to set up empirical calibration curves for the desired gases. In such a case, it has proved convenient to render the pressure-broadening effect of the foreign gases constant by using a constant total pressure for the sample while constructing the calibration curves and obtaining the data for unknown samples. Using this procedure, very satisfactory determinations may be made of methane, sulfur dioxide, carbon dioxide, and carbon monoxide (*20*). The calibration curves for the latter three may be seen in Figure 4. Similar methods for these and other gases subject to pressure broadening have been published (*41, 56*).

Analysis of Unassociated Materials in the Liquid Phase. Mixtures of unassociated liquids may be analyzed in the same straightforward manner utilizing simultaneous equations as used for gas analysis. The term "unassociated liquids" is used to specify materials that are not subject to intense, short-range forces which give rise to the formation of complexes such as those found for hydrogen bonding. In this respect the hydrocarbons are very inert and very easily handled. In practice, when I and I_0 of Equation 2 are determined by a cell-in-cell-out procedure we have:

$$D = \log I_0/I = bc + k \tag{4}$$

where k, the cell attenuation factor, has its origin in the absorption and reflection of the light by the cell windows. In gas analysis it is easy to eliminate k from the equations by obtaining D for the evacuated cell. However, this cannot be done for a liquid cell because of the large changes in reflectivity between an empty and filled cell. If the analyses are made with the desired compounds in a transparent solvent, k can be evaluated by examining the cell filled with solvent. However, the use of solvents is often not practical when certain mixtures such as paraffins are analyzed. Here the calibration is done with the pure materials in the cell and data are obtained on the mixtures with the same cell. The use of the same cell allows an algebraic elimination of the k values.

Let us consider the optical density at λ_1 for the first of three compounds for which calibration data are being obtained for a ternary mixture. With the pure material in the cell we have:

$$\bar{D}_{11} = b_{11}C_1 + k \tag{5}$$

where $\bar{D}_{11}$ is the measured optical density and b_{11} is the true calibration coefficient. Because $C_1 = 1.00$ for the calibration we have:

$$b_{11} = \bar{D}_{11} - k \tag{6}$$

When the optical density, D_1, for the ternary mixture is measured at the same wave length we have:

$$D_1 = b_{11}C_1 + b_{12}C_2 + b_{13}C_3 + k \tag{7}$$

If we substitute the results of Equation 6 and similar ones, we obtain:

$$D_1 = \bar{D}_{11}C_1 + \bar{D}_{12}C_2 + \bar{D}_{13}C_3 - (C_1 + C_2 + C_3)k - k \tag{8}$$

However, because $C_1 + C_2 + C_3 = 1.00$ we have:

$$D_1 = \bar{D}_{11}C_1 + \bar{D}_{12}C_2 + \bar{D}_{13}C_3 \tag{9}$$

Thus it is possible to get sets of linear equations which may be solved for the C_i's without actual evaluation of the attenuation factors.

This method has been used with considerable success for the analysis of mixtures of paraffins boiling in the gasoline range. Unless the sample is from a rather special process which involves only a few materials, it will contain many compounds over an extended boiling point range. Direct infrared examination of such a sample is not practical. Rather the material should be fractionated into a series of cuts containing on the order of five or less compounds each. The choice of wave lengths then follows the procedure used in gas analysis. In Figure 5 may be seen the recorded spectra and three suitable absorption bands for a ternary

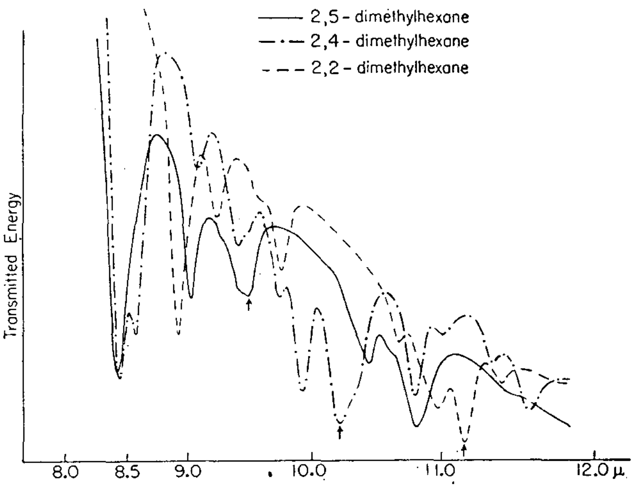


Figure 5. Spectra of Three Dimethylhexane Isomers (*15*)

Curves are tracings of automatically recorded spectra

Table II. Comparison between Blended and Analyzed Composition of Paraffin Mixtures

Compound	Synthetic, %	Calculated, %	Difference, %
n-Heptane	22.2	21.8	−0.4
Methylcyclohexane	27.8	27.8	0.0
Methylcyclopentane	50.0	50.4	+0.4
2,5-Dimethylhexane	22.2	21.5	−0.7
2,4-Dimethylhexane	22.2	22.5	+0.3
2,3,4-Trimethylpentane	22.2	22.8	+0.6
2,3,3-Trimethylpentane	11.2	11.3	+0.1
2,2,3-Trimethylpentane	22.2	21.9	−0.3

mixture of dimethylhexane isomers. As the spectra for such materials are generally rich in bands, it is usually easy to satisfy the condition of optical discrimination. Table II shows some typical results when synthetically blended mixtures have been analyzed by this technique.

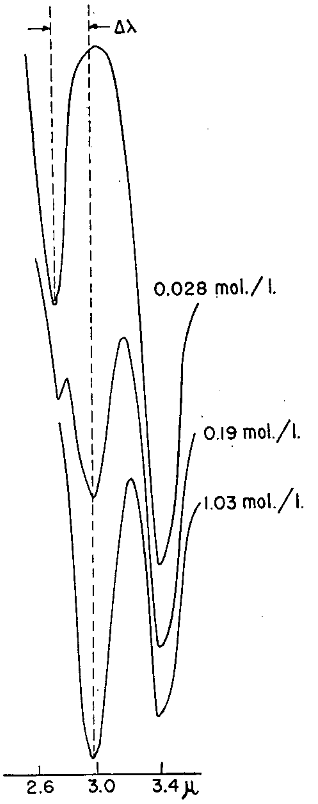


Figure 6. Infrared Spectra in Hydroxyl Absorption Region of Various Concentrations of Ethyl Alcohol in Carbon Tetrachloride Solution *(14)*

Although paraffins may be readily analyzed by the above technique in a cell on the order of 0.006 inch thick, it is not so easy to handle olefins and many polar compounds by the same method, because of the much greater intensity of absorption displayed by the latter materials. In order to get reliable quantitative data, it is often necessary to work with the material in solution. Although this simplifies the cell attenuation factor problem, it introduces new steps into the procedures and also new problems associated with the solvent. Because all solvents are themselves chemical compounds, each will display its own infrared spectrum. The absorption bands of the solvent will interfere with the absorption bands of the solute unless the solvent is transparent in the special region of interest. Although it is not possible to choose a solvent that is transparent throughout the entire infrared region, it is possible to choose a series of solvents with overlapping regions of transparencies, so that investigations over the whole region can be made *(61)*. Cyclohexane and carbon disulfide are favorite solvents for analytical work, as they are both transparent in the region wherein is found the greatest abundance of bands suitable for analytical work.

Although the applications of infrared and Raman spectra for the analysis of nonassociating liquids are parallel, there are some advantages unique to each by virtue of the fundamental processes involved. Whereas the optical density of an infrared absorption band varies exponentially with the concentration of the absorbing component, the intensity of a Raman line varies linearly with the concentration. This makes the infrared method more suitable for measuring low concentrations and the Raman method more suitable for measuring concentrations near 100%. Although a recent publication describes the use of Raman spectra for the determination of olefins *(34)*, the technique has probably been utilized most, to date, for the analysis of aromatics *(28, 55)*.

As long as the materials involved do not associate and there are not too many components in each sample, there is no fundamental limitation to the infrared analysis. If weak and strong absorbers are present together, it may not be possible to measure the former with an accuracy comparable to that possible for the latter. Usually some auxiliary technique like bromination may be called upon to alleviate the situation. In many cases it is not possible to predict reliably whether or not a system is subject to association, chemical interaction, or other interatomic effects whereby Beer's law is violated. In such cases, it is necessary to make detailed tests in order to determine the behavior. As a general rule, trouble of this type may be expected to occur for any system which is known to contain compounds that will form complexes such as hydrogen bonding. Dilute solutions, whereby the average intermolecular distance negates the association effects, may sometimes be used *(55)*. This, unfortunately, often introduces problems of solvent transparency.

Analysis of Liquid Materials Subject to Association. The term "association" in the liquid phase is used here in a very general sense, to specify any phenomenon which is responsible for severe deviations from Beer's law. A common cause of such deviations in the liquid phase is the perturbation effects due to strong electric fields. When two molecules hydrogen-bond, for example, there is a very strong attraction between the dipoles resident in the hydroxyl groups. The effect of the dipole field of one hydroxyl group on the other is such as to shift the resonant frequency and to enhance the intensity of absorption *(28)*. Actually, some of the phenomena responsible for the deviations from Beer's law are neither understood nor have as yet been thoroughly investigated. On one hand, this is a discouragement to the use of infrared and Raman methods for the treatment of such systems, while on the other the very fact that the phenomena manifest themselves in photometric anomalies makes it possible for spectroscopic methods to aid abundantly in their clarification. Thus the research necessary to apply these methods is yielding a rich return in fundamental understanding of some of the chemical and physical interactions that occur between molecules in the liquid state.

Hydrogen bonding is one of the most commonly known forms of association. When it occurs, the absorption due to the hydroxyl group is drastically altered. This may be seen in Figure 6. Here are given the spectra in the hydroxyl region of three different concentrations of ethyl alcohol in carbon tetrachloride solution.

In the 0.028 mole per liter solution may be observed a band at about 2.76μ which is due to the free or unassociated hydroxyl group. At such concentrations the free band is observed, as the average intermolecular distance does not permit hydrogen bonding. In each curve the absorption band at 3.4μ is due to C—H stretching vibrations. For 0.19 mole per liter it is observed that the intensity of the free hydroxyl absorption band has decreased and that a new band at about 3.0μ has appeared. For 1.03 moles per liter the free hydroxyl band has disappeared and the 3.0μ band has become very strong.

The interpretation is that as a molecule becomes hydrogen-bonded the effective force constant for the hydroxyl valence bond is weakened. This weakening results in a shift of the resonant frequency to a lower value; hence the absorption band shifts to a longer wave length. In addition to this change there is also a great enhancement of intensity of absorption. It is clear that in the face of such changes as these, the straightforward application of Beer's law on the hydroxyl absorption band for analytical purposes would be useless. Such difficulties may, however, be circumvented by the use of a solvent such as carbon tetrachloride and a thick cell wherein the material is examined in such low concentration that association does not occur. Very accurate determinations may thus be made of individual phenols and alcohols in the presence of nonhydroxylated materials and of total hydroxyl content in group-type analyses.

An example of another effect, not so clearly understood, is the interaction that occurs between alcohols and ketones with an alteration of the spectra of the latter *(54)*. In Figure 7 is plotted the optical density of methyl ethyl ketone at two different wave lengths as a function of ethyl alcohol content. The concentration of methyl ethyl ketone is constant throughout and a correction is made at each point for the absorption due to the ethyl alcohol. It is clear that Beer's law is not followed, for a constant concentration of the ketone would then yield constant optical density values. At one wave length the ethyl alcohol enhances the optical density, whereas it decreases it at another. The 5.82μ wave length is the carbonyl stretching band. This band might be expected to be affected by association between the carbonyl groups and the

Table III. Comparison between Analyses of a System of Oxygenated Compounds with and without Sodium Carbonate Treatment

	Untreated Sample			Treated Sample		
	Known, %	Observed, %	Diff., %	Known, %	Observed, %	Diff., %
Propionaldehyde	40	31.0	−9.0	40	37.8	−2.2
Methanol	30	33.7	+3.7	20	19.0	−1.0
Acetone	30	35.3	+5.3	40	43.2	+3.2
Average difference			6.0			2.1

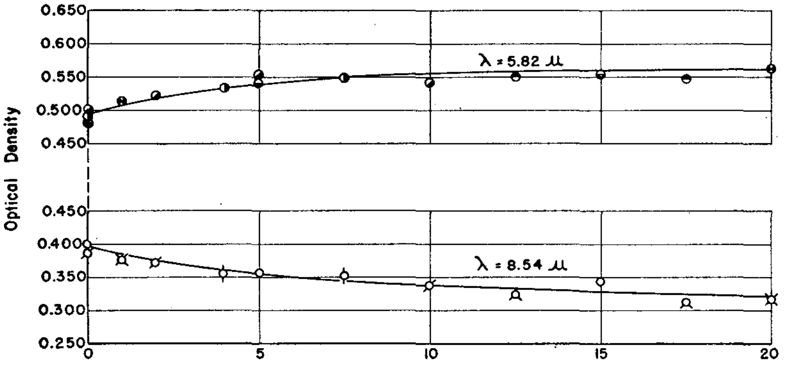


Figure 7. Effect of Concentration of Ethyl Alcohol on Optical Density of Constant Concentration of Acetone at Two Wave Lengths of Maximum Absorption

Methyl ethyl ketone. Solvent, carbon disulfide. 0.006-inch cell

hydroxyl groups of the ethyl alcohol. Such interactions have been reported and studied (*30, 31*). However, in the present case two factors indicate that the effects are not due to such association. One is that the wave length of maximum absorption for the carbonyl band does not change. Any association phenomenon strong enough to affect the intensity of absorption of a group vibration would be expected to change the resonant frequency. Another factor is that the 8.54μ band absorption decreases as the ethyl alcohol concentration increases, which is contrary to the expected behavior for a frequency perturbed by a hydrogen-bonded complex. The results shown in Figure 7 are typical of those obtainable for a number of combinations of ketones and alcohols.

In view of the slight acidity of alcohols it was postulated that the effect was of the nature of a keto-enol equilibrium disturbance due to a change in ionic balance. Specific experiments have borne out the dependence of the effect upon the types of ions present. Accordingly, the calibration and sample systems were all made basic with an inorganic material. The effects of such treatment may be seen in Table III. Here are given the results of the analysis of a synthetic blend containing propionaldehyde, methanol, and acetone which was handled by the method described above for unassociated liquids. In addition are given the results for the analysis of the sample wherein the solutions were rendered basic in each case. It is seen that there is a great reduction of the differences between observed and calculated concentrations. Even with the treatment these differences are large. Nevertheless, they are satisfactory for some purposes and in view of the other undesirable phenomena that occur in such a mixture it is perhaps remarkable that an analysis is feasible. Other known phenomena occurring are: the formation of acetals by reaction between the alcohol and aldehyde, hydrogen bonding, and differential evaporation rates.

The latter phenomenon can be troublesome in systems containing the low molecular weight alcohols in small concentrations. It is well known that the high boiling points of the simpler alcohols are due to the intermolecular attraction hydrogen-bonding forces (*48*). When the alcohols are present in small enough concentrations, these forces are inoperative and the relative rate of escape through evaporation is hastened. Hence, it is difficult to maintain a fixed level of concentration and special techniques are called for in the calibration and handling of such systems. A manifestation of this effect may be seen in Figure 8, in which are plotted the optical densities at 9.7μ, a characteristic band for methanol, for three different series of samples.

In each case the methanol concentration was constant and the percentage of acetone varied. In the top curve it is to be seen that the points scatter very much. These samples were prepared under ordinary conditions, with the materials being mixed by shaking in glass-stoppered bottles. As the scattering was ascribed to uncontrolled evaporation losses, the data for the middle curve were obtained by mixing the samples in cork-stoppered bottles. A definite improvement is observed. As a means of decreasing the scattering, the samples were next mixed in cork-stoppered bottles which were filled to the top and had glass beads added to provide agitation. This gave the data seen in the bottom curve. Two things are clear from this: the importance of a very careful handling technique and the fact that acetone has no association effects on the optical density of methanol at 9.7μ.

As further applications of molecular spectroscopy are made, the study of the more difficult systems and the development of methods for handling them will add much to the physical chemistry of liquid mixtures, particularly organic mixtures. In fact, a full understanding of some of the phenomena will be necessary before generalized analytical techniques can be outlined.

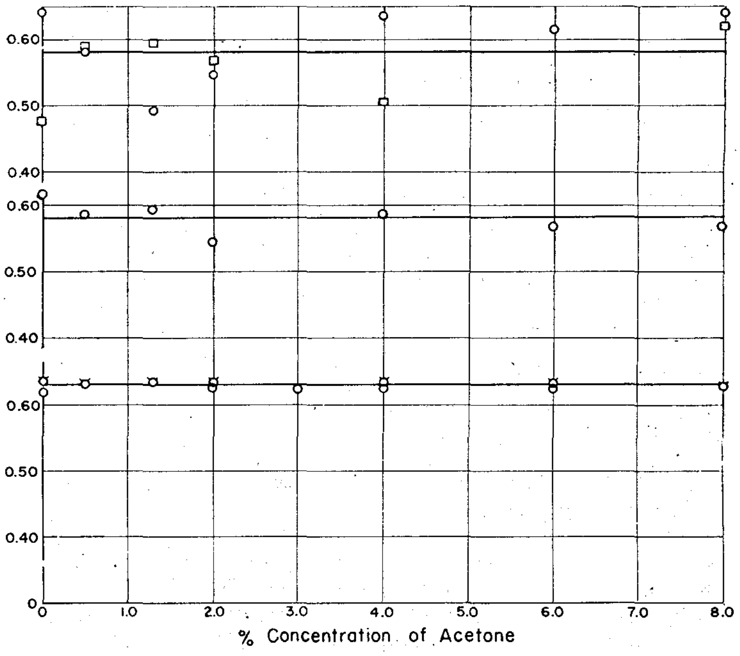


Figure 8. Optical Density of Constant Concentration of Methanol Plotted against Concentration of Acetone Present

Three experimental techniques of mixing

Group-Type Analyses. In group-type analyses the aim is to determine by a minimum number of observations the molal concentration of a particular atomic grouping such as hydroxyl, carbonyl, carboxyl, etc., in a sample. Two factors combine to make such analyses possible by infrared and Raman spectroscopy. One is the fact that, in the absence of molecular association, the absorption frequency of a particular group is essentially independent of the remainder of the molecule. This makes it possible to take data in a small wave-length span and to know that there will be a contribution to the absorption or to the Raman scattering from the same group in each molecular species present. The other factor is that the absorption per atomic group is approximately constant for all molecules containing the group.

Table IV. Determination of CH_3, CH_2, and Aromatic CH in Blends of Single-Ring Aromatics (*35*)

Aromatic Blend No.	No. of CH_3 Groups Found	True	Diff.	No. of CH_2 Groups Found	True	Diff.	No. of Aromatic CH Groups Found	True	Diff.
A-1	1.02	0.99	0.03	0.35	0.33	0.02	5.09	5.16	−0.07
A-2	1.29	1.25	0.04	0.58	0.65	−0.07	4.99	4.85	0.14
A-3	1.64	1.46	0.18	0.88	0.98	−0.10	4.76	4.70	0.06
A-4	2.12	2.00	0.12	0.51	0.69	−0.18	4.23	4.29	−0.06
A-5	1.66	1.58	0.08	0.83	0.89	−0.06	4.61	4.51	0.10
Av. error in number of groups			0.09			0.09			0.09

This application may be said to have had its start in the early work of Rose at the National Bureau of Standards (*51*, *52*). Working in the overtone region between 1.2 and 1.8μ, he showed that the contributions to the optical density of the various functional groups on a molal basis are nearly constant in different hydrocarbons. Work in the overtone region has recently been extended by Hibbard and Cleaves (*35*), who have developed a method whereby the CH_3, CH_2, and aromatic CH concentrations may be determined in hydrocarbon samples. In this, they located characteristic bands in the 1.10 to 1.25μ region at which molal absorption coefficients were determined for each of the groups. In Table IV may be seen the results of applying their method to a series of known blends containing single-ring aromatics. The results are in terms of average number of groups per molecule and the agreement between calculated and observed values is remarkably good.

The invariancy of the absorption coefficient of a particular atomic group throughout a series of compounds may be seen from Table V, which gives the hydroxyl absorption coefficient for a series of alcohols and of phenols. The units in each case are arbitrary, as they depend upon cell thickness. For the alcohols the coefficients have been determined for concentrations in terms of moles per liter and for the phenols in terms of moles per gram.

Table V. Absorption Coefficients for Free Hydroxyl Band for Alcohols and Phenols

Alcohol	Coefficient	Phenol	Coefficient
Ethyl	62.4	Phenol	3.10 × 10^5
n-Propyl	65.9	*o*-Cresol	2.94 × 10^5
n-Butyl	64.8	*p*-Cresol	3.19 × 10^5
n-Amyl	62.8	2,4-Di-*tert*-butylphenol	3.02 × 10^5
n-Octyl	68.6	2,4,6-Tri-*tert*-butylphenol	2.89 × 10_5

Table VI. Comparison between Known Hydroxyl Content of Blended Samples and Observed Concentrations

Alcohol	Sample 1	Sample 2	Sample 3
		Moles per Liter	
Ethyl	5.7		3.4
n-Propyl	4.5	2.7	2.7
n-Butyl	3.6	2.2	2.2
n-Amyl	...	3.7	1.8
n-Octyl	...	1.2	1.3
Known hydroxyl	13.8	9.8	11.4
Observed hydroxyl	13.4	9.6	11.1
Error	0.4	0.2	0.3

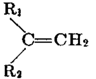
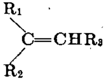
These data were obtained at the 2.76μ band (the free hydroxyl) in carbon tetrachloride solution and at concentrations below that necessary for hydrogen bonding.

The accuracy whereby such data may be applied to a determination of actual molal concentrations may be seen in Table VI, which gives the composition of blends containing alcohols and the concentrations calculated from infrared data.

Anderson and Seyfried (*2*) have recently reported extensive application of group-type analysis to the determination of alcohols, esters, carboxylic acids, ketones, and five different olefin types in hydrocarbon synthesis naphthas. The wave lengths (with the exception of the one for hydroxyl groups) they used are given in Table VII. Investigations of group-type analyses are being pursued concurrently in a number of places and future developments may be expected to yield increased precision of methods, more extensive applications, and a fuller understanding of the dependence of the second-order variations of the absorption coefficients on the remainder of the molecules. The Raman scattering coefficients are also invariant and an application of them for the determination of total olefin and aromatic content has recently been reported (*34*).

Molecular Structure Determination. It is possible in principle to derive mathematically the vibrational modes and frequencies of any molecule wherein the interatomic distances, bond angles, and force constants are known. However, this process is much too difficult and laborious for all but the simplest of molecules, and hence, spectra and known structure of most molecules must be correlated on a semiempirical basis. The converse of this correlation is of interest in molecular structure determination—i.e., to determine from an observed spectrum the structure of the material examined. In the application of infrared and Raman spectra this is done almost entirely by the use of diatomic and group-type frequencies.

Table VII. Characteristic Band Positions for Various Functional Groups (*2*)

Functional Group	Wave Length, μ
CHO (aldehydes)	3.63 ± 0.01
COOH (acids)	3.82 (broad band)
COO (esters)	5.71 ± (0.01)[a]
CO (ketones)	5.78 ± (0.01)[a]
—O— (acetals and ethers)	8.8
Olefinic Group	
$RCH{=}CH_2$	10.05, 10.98 ± (0.02)
 $R_1R_2C{=}CH_2$	11.24 ± (0.02)
trans-$R_1CH{=}CHR_2$	10.36 ± 0.02
cis-$R_1CH{=}CHR_2$	14.0–14.6 (variable)
 $R_1R_2C{=}CHR_3$	11.9–12.7 (variable)

[a] Except for first few members of series.

In Equation 1 was given the vibrational frequency of a diatomic molecule in terms of the force constant and the reduced mass. This is applicable as a first approximation to the valence bond vibrations of diatomic groups in more complicated molecules. This rests upon the fact that such vibrations are roughly independent of the remainder of the molecule. Hence, all molecules containing CH groups will absorb in the neighborhood of 3.4μ, all molecules containing C═O groups will absorb in the neighborhood of 5.7μ, etc. This, of course, allows the empirical assignment of absorption bands to various groups on the basis of the spectra of homologous series. Once these assignments are established, they may be used in the examination of materials of unknown structure to determine the presence or absence of specific groups. In Table VIII may be seen a number of groups and the frequencies or wave lengths at which Raman scattering or infrared absorption is observed.

Table VIII. Fundamental Stretching Vibration Frequencies of Diatomic Groups

Group	Frequency, Cm.$^{-1}$	Wave Length, μ	Group	Frequency, Cm.$^{-1}$	Wave Length, μ
O—H	3680	2.7	C═O	1740	5.7
C—H	2920	3.4	C—O	1030	9.7
N—H	3350	3.0	C≡N	2090	4.8
C≡C	1970	5.1	C═N	1650	6.0
C═C	1620	6.2	C—N	1050	9.5
C—C	990	10.1	S—H	2600	3.6

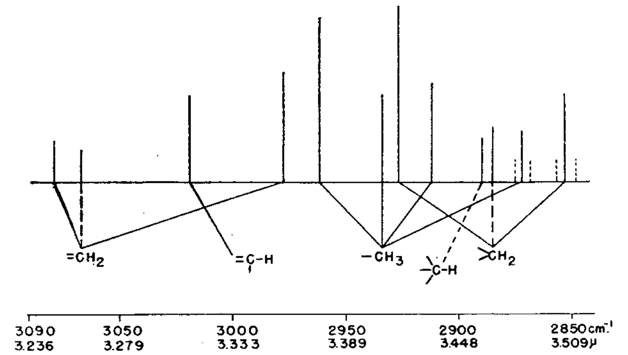


Figure 9. Infrared Absorption Frequencies Observed for Various Types of Carbon Hydrogen Groups in 3.4μ Region (55)

Based on data from (29)

In addition to these values the ones given in Table VII and as used for quantitative group-type analysis may be used, particularly for the determination of olefin substitution. The wave lengths of Table VIII are meant to represent the approximate centers of the spectral regions in which the different groups are observed. There may be considerable variation in how a particular group type absorbs. Although this may be a disadvantage in some cases, owing to overlapping of regions, it is an advantage in others in that it allows a further classification. For example, Barnes *et al.* (3) have shown absorption frequencies for five different types of compounds containing carbonyl groups—i.e., anhydrides, esters, acids, ketones, and aldehydes. Regularities, according to class, are observed which allow in some cases an assignment of a compound observed to contain a carbonyl group into one of the five classes. They have similarly discussed the aliphatic C═C and the aromatic ring frequencies. From the same laboratory has come one of the most complete assignment charts published (4). It gives not only the frequency values for almost all groups that may be reliably recognized by this technique, but the ranges of variation as well. Among the groups that may be identified by their data are those of Table VIII, and various types of aliphatic C—H groups, of phenyl ring substitution, of olefin substitution, etc.

An example of the variation observed for a particular diatomic group type may be seen in the work of Fox and Martin (29). They found in a study of hydrocarbons in the neighborhood of 3.4μ that the spectra could be classified according to whether the C—H group was one of the following types: $=CH_2$, $=\underset{|}{C}-H$, $\overset{\diagdown}{-CH_3}$, $-\overset{\diagdown}{\underset{\diagup}{C}}-H$, and $\overset{\diagdown}{\underset{\diagup}{C}}H_2$. A diagram constructed from their data may be seen in Figure 9. Here the dashed lines indicate frequencies that are not always observed. Such details of structure are of tremendous importance in some structure problems, in that by the use of them alone it is sometimes possible to choose between several alternative structures. The detail of structure illustrated by Figure 9 is not resolved by the ordinary rock salt infrared spectrometer, but required either a grating instrument or the use of a prism of highly dispersing material such as lithium fluoride. It is expected that the further study of CH structure detail in molecules other than hydrocarbons will yield rules of very extensive use in the examination of other classes of organic materials.

Probably the most important single factor in the application of molecular spectra to problems of structure is experience. Fortunately, this may often be obtained by a gradual process, whereby the worker is able to learn and gain confidence by the recognition of the more easily established groupings. As his proficiency advances he must draw upon other sources of information than the mere location of absorption bands. He must know the selection rules whereby certain vibrations are forbidden in infrared spectra and others in Raman spectra. With the use of judgment, gained through experience, the investigator can often utilize to good advantage the shapes and intensities of the observed bands. The invariance of the intensities as used above in group-type analysis is often an aid in deciding whether a molecule may be singly or doubly substituted with a particular group. The information that may be supplied by the chemist as to the most probable structure from the chemistry involved is often invaluable, as it provides the initial direction of thought. The use of auxiliary chemical treatments whereby specific groups are either removed or added in preferred positions is often extremely useful. At the present time, infrared dichroism is very promising as another auxiliary technique (25). The use of recently developed selenium polarizers, either transmission or reflecting, makes the examination of materials with polarized infrared radiation practical. Differences of intensities of specific bands as a function of the angle of the electric vector of the incident radiation relative to the sample allow the determination of the orientation of the absorbing groups.

ULTRAVIOLET ABSORPTION SPECTROSCOPY

When radiant energy in the ultraviolet part of the spectrum, roughly 2000 to 4000 A., is absorbed by a material, the energy is utilized for the excitation of the electronic energy levels. These involve the displacement of the valence bonding electrons and

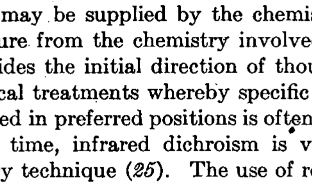
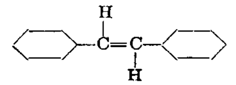


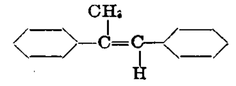
Figure 10. Steric Hindrance to Resonance between Conjugated Chromophores (42)

1.



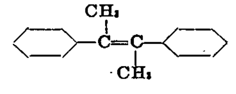
Stilbene

2.



Monomethylstilbene

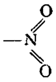
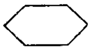
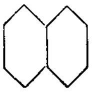
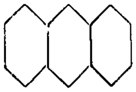
3.



Dimethylstilbene

the energies, of the order of 100 kg.-cal. per mole, are very much higher than those involved in the excitation of molecular vibration and rotational frequencies. The exact theoretical description of the electronic energy levels and associated phenomena is necessarily in the language and methods of quantum mechanics (*43*, *45*), but for practical applications this approach is too difficult and complex for the usual analytical chemist. It is, therefore, very fortunate that there exists an empirical and descriptive theory which deals with the ultraviolet absorption in terms of absorption ascribable to specific atomic groupings. This theory, which might be called the chromophore theory, has been excellently described by Lewis and Calvin (*42*) and recently reviewed by Ferguson (*27*) and others (*15*).

Table IX. Chromophoric Groups and Characteristic Wave Lengths

Group	Wave Length, mμ
C=O	280
—S—H	228
—N=N—	350
	366
	265
	311
	475

In this theory, the electric vector of the electromagnetic radiation induces oscillations of the valence electrons. Hence the more mobile or loosely bound are the electrons, the lower the frequency of absorption. This relates the absorption very closely to resonance, as resonating structures allow a greater mobility of the binding electrons than do saturated systems. Indeed, it is found that, in general, those materials known to possess resonance will absorb in the ultraviolet and visible spectral regions, whereas the nonresonating compounds are essentially transparent. An example of such a comparison is between the aromatics and the paraffins. The aromatics which possess the well-known resonance characteristics of the benzene ring possess characteristic ultraviolet absorption, whereas the paraffins are transparent throughout the visible and the ultraviolet down into the vacuum ultraviolet region below 2000 A.

Empirical studies have shown that the ultraviolet absorption may be ascribed to certain atomic groupings called chromophores. An example is the benzene ring. All compounds containing it will possess absorption somewhere in the vicinity of 250 to 280 mμ. Furthermore, in a homologous series such as the alkylbenzenes, for example, the spectra will all be very similar in spectral location, shape, and intensity. Such data show that the absorption is due to the unsaturation and resonance effects inherent in the benzene ring and is essentially independent of the alkyl substituents. Several common chromophores and the wave lengths characteristic of their absorption bands may be seen in Table IX. These wave lengths are not exact, but rather serve to locate the region of absorption.

The fact that the absorption is governed by the chromophores within the molecule results in ultraviolet spectra having distinct general properties in comparison to infrared and Raman spectra. For the latter two, a unique spectrum is found for each different compound, whereas for the former the materials absorb according to class, which in this case is determined by the chromophoric constitution. This is both an advantage and a disadvantage. In analytical applications it means that the multicomponent analysis of systems of similar materials on the basis of their ultraviolet spectra is often not feasible. However, it is often possible to examine a mixture of several compounds and readily determine the concentration of a particular class.

When two chromophores are conjugated, the over-all resonance of the molecule is increased. The combination exhibits not only the absorption frequencies characteristic of the individal chromophores, but also a new and lower frequency. The situation is somewhat analogous to the joining of two segments of vibrating string. The new segment comprising the original two will possess a lower fundamental vibration frequency. Needless to say, this phenomenon of decrease of frequency with conjugation is a most valuable tool in determination of molecular structure. If there exists a condition which will tend to inhibit the resonance of the two chromophores together, the spectrum will revert toward that which is a mere superposition of the spectra of the individual chromophoric groups. An example of this may be seen in Figure 10.

In curve 1 is given the spectrum of stilbene. The low frequency band is due to the conjugation of the phenyl rings through the olefin group. In curve 2 is given the spectrum for monomethylstilbene. It is seen that the corresponding band has shifted to a higher frequency, as a result of the steric hindrance of methyl group to the coplanarity of the two phenyl rings. Curve 3 gives the spectrum for dimethylstilbene, and the effect is demonstrated to a greater degree—i.e., the two methyl groups inhibit the coplanarity of the two structures with a large resultant shift to higher frequencies. Such observations are at times very significant in locating the positions of substitutions relative to resonating structures.

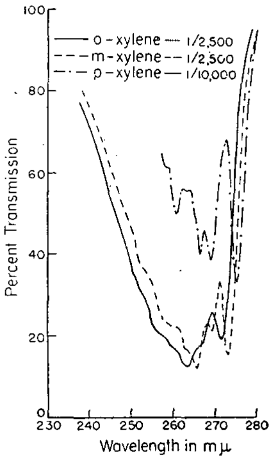


Figure 11. Ultraviolet Absorption of *o*-, *m*-, and *p*-Xylenes

Figures give dilution factors for materials in iso-octane solution (*15*)

Analytical Applications. Most quantitative analytical applications of ultraviolet absorption are based on Beer's law of absorption as given in Equation 2. Deviations from Beer's law are not so frequently encountered in ultraviolet absorption as in infrared absorption. One reason for this is that the energies involved in electronic transitions are much greater than those involved in vibrational transitions and, hence, are less affected by low energy intermolecular interactions. Several notable exceptions do occur, however. Because materials of a given class possess very similar spectra, ultraviolet absorption is not a tool of wide applicability for the analysis of multicomponent mixtures of similar compounds.

Exceptions to this occur, of course, and one of particular importance is the analyses of mixtures for the light aromatics: benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, and *p*-xylene. Although these all possess the phenyl ring chromophore absorption, there are second-order differences due to the different substitutions. These are sufficient to allow choice of wave length for each compound and the use of simultaneous equations as described above. An idea of the similarity of spectra may be attained from Figure 11, which gives the per cent transmission plotted against wave length for the xylene isomers. The numbers give the dilution ratios, and it is noted that the *p*-xylene

Table X. Comparison between Blended and Observed Aromatic Concentrations (*15*)

Compound	Blended, %	Observed, %	Difference, %
Benzene	7.7	7.4	−0.3
Toluene	15.4	15.1	−0.3
Total aromatics	23.1	22.5	−0.6
Benzene	0.5	0.6	+0.1
Toluene	2.0	2.1	+0.1
Ethylbenzene	0.5	0.3	−0.2
Total aromatics	3.0	3.0	0.0
Ethylbenzene	0.5	0.6	+0.1
o-Xylene	1.5	1.3	−0.2
m-Xylene	4.0	4.5	+0.5
p-Xylene	4.0	4.2	+0.2
Total aromatics	10.0	10.6	+0.6

absorbs with approximately twice the intensity of the others. This is a general characteristic of para substitution. Because of the very intense absorption displayed by most absorbing materials it is necessary to use rather dilute solutions in such nonabsorbing solvents as iso-octane or other paraffins when working with absorption cells with thicknesses of the order of 1 cm. This is the standard cell thickness for the Beckman quartz spectrophotometer (*12*), which is without doubt the most widely used instrument of this type today. Working in such dilution ranges calls for careful operation and cleanliness of glassware, cells, etc.

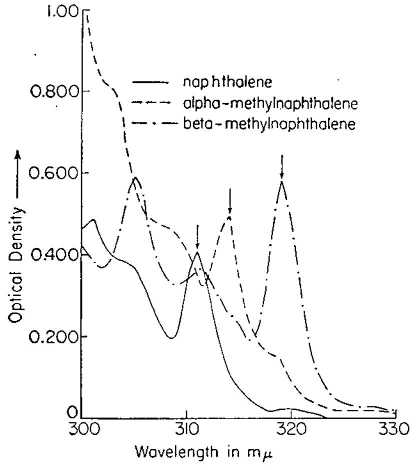


Figure 12. Ultraviolet Absorption Spectra (*16*)

Although wave lengths may be chosen for each of the six aromatics named, the differences are not great enough to give good optical discrimination. As a result, it is necessary that a sample be subjected to distillation to reduce the number of aromatics in any one sample. This can be done by making a benzene cut, a toluene cut, and a xylene cut, each of which is analyzed for the compounds named plus those boiling nearby. In this manner, satisfactory accuracy is achieved. Typical results may be seen in Table X, which compares the blended and observed concentrations for several synthetic samples.

Individual concentrations of the heavier alkylbenzenes cannot be determined without severe fractionation, because of the multiplicity of the higher aromatics and their closely grouped boiling points. It is possible to make several cuts through the C_9 aromatic range and by suitable groupings to get satisfactory total aromatic content. Satisfactory determinations of naphthalene in mixtures boiling in the kerosene range may be made by use of the characteristic absorption of the naphthalene chromophore (*1, 13, 16*). This absorption occurs in the 300 to 320 mμ range, in which the mononuclear aromatics possess no characteristic bands. In samples containing high concentrations of the mononuclear aromatics, there will be a certain amount of background absorption. Suitable correction may be made for this by the use of the shapes of the absorption bands. In somewhat higher boiling cuts naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene may be determined simultaneously. The spectra for these three compounds may be seen in Figure 12. Here again we have the situation of the absorption in each case being due to a common chromophore and yet there being enough differences to allow a multicomponent analysis. The arrows in Figure 12 indicate the wave lengths used for calculation, and the concentrations are determined by a set of simultaneous equations based on Beer's law of absorption as described above. Ultraviolet absorption is readily extended for the calculation of the anthracene and phenanthrene concentrations. In each case, the absorption is due to a chromophore formed by several condensed ring aromatics. Total concentrations of dimethyl and ethylnaphthalenes may be reliably estimated, but the calculation of the individual components is not practical without severe fractionation.

The localization of the absorption in specific chromophores is responsible for one of the most distinguishing features of analysis by ultraviolet absorption, especially in contrast to infrared and Raman spectroscopy. It often allows the determination of one or a class of compounds in a mixture independent of the remainder. During the war it was used extensively for the determination of butadiene (*6, 53*) in C_4 hydrocarbon fractions. This is possible because of the conjugated diolefin chromophore, which has an onset of absorption at about 235 mμ. The same chromophore makes possible the determination of pentadienes (*47*), hexadienes, etc. Many other examples could be cited wherein the absorption due to a chromophoric group is utilized to determine one compound or class of compounds in a mixture. The carbonyl chromophore allows the determination of ketones in the presence of other organic materials. Distilled water is very transparent throughout the ultraviolet region and makes an excellent solvent when it can be used. The simple alcohols are also transparent and suitable for solvents. In going to the higher alcohols, one may encounter impurities in the form of organic acids which render the material unsuitable, owing to the heavy absorption by the carboxyl group in the shorter wave lengths.

Other examples include the determination of styrene in hydrocarbon mixtures. Because of the conjugation of an olefin group, which as a true chromophore possesses absorption at about 190 mμ, with the benzene ring a new band near 290 mμ appears. This allows a precise determination of styrene in the presence of alkylbenzenes. Furfural may be determined in gas oils, although in such a case there may be considerable absorption due to the oil itself. To correct for it suitably may necessitate an examination of the furfural-free oil. By virtue of the phenyl ring absorption and the variations due to substituent changes, it is possible to determine phenol and the isomeric cresols (*50*) and aniline, *N*-methylaniline, and *N*,*N*-dimethylaniline (*62*).

In the chemistry of materials from natural sources the technique of ultraviolet absorption has been very valuable, particularly in the study of the fatty acids. Brode and co-workers (*10*) have described a method of determining the amount of two, three, and four double bond conjugation in the presence of nonconjugated unsaturated fatty acids. Other workers have reported an ultraviolet absorption method for the determination of polyunsaturated constituents in fatty materials (*9*), a relationship between unsaturation and ultraviolet absorption spectra of various fats and fatty acids (*5*), and data for isomerized arachidonic and linolenic soaps in fatty materials. Ultraviolet absorption has also been used for the determination of vitamin A in margarine (*44*), and in the study of the changes during oxidation of vitamin A oils (*33*). A spectrophotometric method for the determination of α-eleostearic acid in tung oil has been described (*46*).

Molecular Structure Determination. Because compounds absorb in the ultraviolet according to chromophores, the technique of ultraviolet absorption is particularly useful in the classification of materials of unknown structure. The spectrum of the unknown material is obtained and regions of maximum absorption are ascertained for comparison with tables of results for known chromophores such as those given in Table IX. This may be sufficient, for example, to determine whether the compound is a mono-, di-, trinuclear, etc., aromatic; whether it may contain the conjugated diolefin structure, a mercaptan group, a nitro group, etc.; or whether it may possess two chromophores arranged in conjugation. It is well known that the conjugation of two chromophores results in the appearance of new bands at longer wave lengths. Ordinarily, the interpretation of the ultraviolet spectra is greatly aided by information available from other sources, such as predictions based on the synthesis of the materials, the history of any treatments, and the results of auxiliary chemical tests. Such information is usually adequate to remove the ambiguity which may result if a material exhibits absorption that may be ascribed to either of two chromophores. This latter is not an unusual situation, because the bands for the more highly substituted materials are generally very broad.

Once the material is classified according to chromophore, further information may be deduced from the shape, intensity, and detailed location of the bands. For example, para substituted aromatics are known to be very strong absorbers in comparison to ortho and meta substituted ones. As the complexity of the substituents increases, the detailed structure of the bands for alkylbenzenes and polar substituted aromatics disappears. The structure of a band—i.e., the appearance of a number of maxima—results from the multiplicity of vibrational transitions that may accompany an electronic transition. An increase of complexity of substitution effectively increases the number of allowable vibrational transitions which may overlap at the expense of the detailed structure. A rather voluminous literature exists on the empirical interpretation of ultraviolet spectra; review articles by Lewis and Calvin (*42*) and by Ferguson (*27*) and the detailed studies of aromatic systems by Jones (*40*) are recommended. As in the case of infrared and Raman spectroscopic studies, the experience of the investigator is of great importance, for he will be able to deduce valuable information from other characteristics, such as shape and size, than the wave lengths of maximum absorption.

The use of various solvent effects is valuable for augmenting the information available from the spectra. By solvent effects are meant the changes in spectra that occur when the material is examined in various solvents. Three of these can be mentioned: ionization, wave-length shifts depending upon localized interaction between the solute molecules and the solvent, and wave-length shifts that are nonspecific in the sense of not depending upon definite, localized atomic groupings in the solute molecules. Many polar substituted aromatics exhibit very large shifts to the red of their absorption maxima when examined in basic or acidic solution. The shifted spectra are really not due to a perturbation of the energy levels of the solute molecules, but are rather the spectra of the ionized materials. In this manner, it may be readily determined whether a substance of unknown or questionable structure is itself acidic or basic in nature by observing which type of solvent produces a large shift of the spectrum. For this type of investigation distilled water to which has been added either an inorganic acid or base is a satisfactory solvent in many cases. As examples of such applications, Ewing and Steck (*26*) have recently utilized the technique in their studies of the acidic and basic properties of quinolinols and isoquinolinols and Irvin and Irvin (*39*) have made similar studies of various 4-aminoquinolines. In addition to indicating whether the solute material is acidic or basic, the behavior of the ion spectra may be utilized to determine substitution.

It has recently been determined (*17*) that the classes of substituted phenols vary a great deal in their activity. The unhindered phenols—i.e., those with small or no substituents on the ortho positions—are relatively acidic. The partially hindered phenols—i.e., those with a bulky substituent such as a *tert*-butyl on one ortho position—are much less acidic, whereas the hindered phenols—i.e., those with both ortho positions occupied by large substituents—are very much less acidic. These behaviors are demonstrated in Figure 13. Here are seen the spectra for one of each type of phenol under various solvent conditions. The concentration of sodium hydroxide which produces a complete shift of spectrum for the unhindered phenol produces an incomplete shift for the partially hindered phenol, and the concentration which produces a complete shift for the latter produces only a partial shift for the hindered phenol. Such results demonstrate the very strong hindering effects of the ortho positions and show how the ionization behavior may be used to gain information concerning the ortho substituents. This ionization behavior allows the same sample to be analyzed for both phenols and aromatics, inasmuch as the spectra for the two are separated by the use of the sodium hydroxide solution.

Another phenomenon whereby the ortho substitution may be assayed is the wave-length shifts attendant to short-range intermolecular interactions. If a simple phenol is examined in ethyl alcohol solution, its spectrum is shifted to the red in comparison to its behavior in a nonpolar solvent such as a paraffin. This shift is known to be due to an interaction between the hydroxyl groups of the solute molecules and the solvent molecules (*19*). When the positions ortho to the hydroxyl group are occupied by large groups such as *tert*-butyl the shift is practically eliminated, because the hydroxyl group of the phenol is hindered from attaining close proximity with the polar solvent molecules. This effect is demonstrated in Figure 14. It is obvious that such data can be used to determine the presence or absence of large ortho substituents in materials of unknown composition.

The other solvent effect mentioned is nonspecific, in so far as it does not depend upon the presence of a particular acidic, basic, or polar group in the solute molecule. It is the result of electronic polarization interactions between the resonant system of the absorbing molecule and the solvent

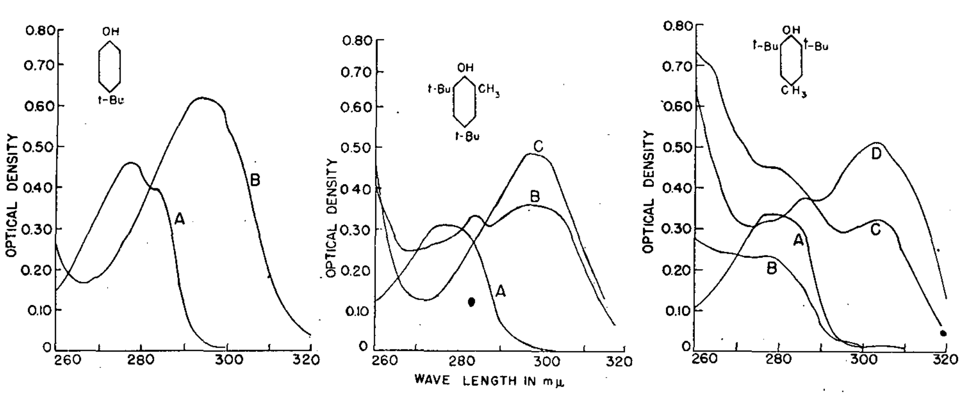


Figure 13. Ultraviolet Absorption Spectra of *p-tert*-Butylphenol, 2-Methyl-4,6-di-*tert*-butylphenol, and 2,6-Di-*tert*-butyl-4-methylphenol (*17*)

A. Ethyl alcohol
B. Ethyl alcohol plus 1.0 × 10^{-1} mole per liter of sodium hydroxide
C. Ethyl alcohol plus 5.0 × 10^{-1} mole per liter of sodium hydroxide
D. Ethyl alcohol plus 5 moles per liter of sodium hydroxide

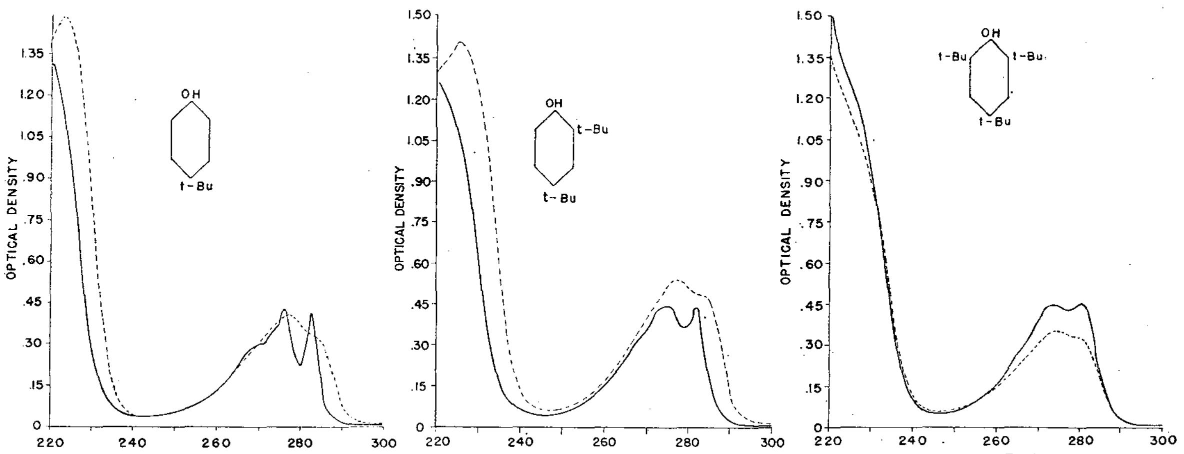


Figure 14. Ultraviolet Absorption Spectra of *p*-*tert*-Butylphenol, 2,4-Di-*tert*-butylphenol, and 2,4,6-Tri-*tert*-butylphenol (*19*)

Solid curve, iso-octane solution; dashed curve, ethyl alcohol solution

molecules. It is strong for the polynuclear aromatics. The effect may be seen in Figure 15, which shows the large wave-length changes observed for naphthacene when examined in benzene as contrasted with the spectrum obtained in iso-octane solution. When precise values for wave lengths of maximum absorption are being obtained, it is therefore necessary to consider the solvent in which the material is being examined.

In the over-all picture, ultraviolet absorption, despite its particular advantages, must be regarded as a somewhat less powerful tool for structure determination than infrared absorption or Raman spectra. One major reason for this is that many compounds possess no ultraviolet absorption at all and the technique is, therefore, powerless for them. The number of known and well established chromophores is fairly small and there is considerable overlapping of the bands observed for them. Of course, the fact that the absorption depends essentially upon the chromophore and not upon the remainder of the molecule imposes limitations on the deduction of detailed information. For best results, the ultraviolet technique should be used in conjunction with infrared or Raman spectroscopy. In this manner, they complement each other and the solution of many problems is greatly simplified.

MICROWAVE SPECTROSCOPY

In the gas phase, molecules possess discrete rotational energies. This was first observed in the fine structure of infrared absorption bands wherein transitions occur simultaneously between vibrational and rotational levels. The energies involved in the rotational states are small and the energy differences between states are so minute that they generally cannot be directly observed with any standard optical equipment. Until the development of radar in World War II, the spectral region in the neighborhood of 1-cm. waves was inaccessible for precise experimental investigations. Therefore, many rotational bands were not discovered and measured until very recently. Since the war, there has been a great burst of activity in microwave spectroscopy in both industrial and academic institutions and there has been continuous progress both in the number of compounds investigated and in improvements of equipment and technique.

In simplest terms, the method consists of passing electromagnetic waves in the microwave region through a sample of material and observing the frequencies at which absorption occurs and the intensities of absorption. The material must be examined in the gas phase because of very severe pressure broadening. This is a result of the fact that satisfactorily to observe transitions involving such small energies, relatively long intervals between collisions are necessary. The source of radiation is a microwave generator which produces monochromatic radiation. The wave length is changed either by changing generators or by changing operating conditions. This is simpler than most molecular spectroscopy work wherein the source generates a spread of wave lengths from which a monochromator isolates a narrow region. The sample being investigated is contained in a wave guide which may be several meters in length and at a pressure of the order of 1 mm. of mercury or less.

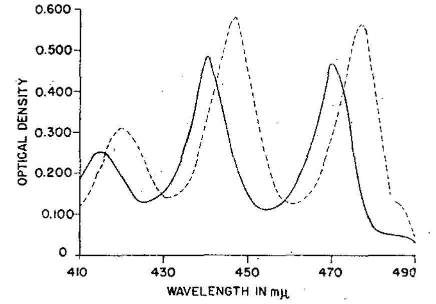


Figure 15. Ultraviolet Absorption Spectra of Naphthacene

Solid curve, iso-octane solution; dashed curve, benzene solution

The rotational absorption frequencies depend only upon the moments of inertia of the molecule. Each absorbing molecule displays a set of lines that is similar to the emission lines from the elements. As the microwave region is broad and excellent resolution is obtainable, the technique looks very favorable for the identification and measurement of specific compounds in mixtures on the basis of individual absorption lines. This has been pointed out by Dailey (*23*), who estimated that, in principle, 1000 different organic compounds could be quantitatively determined from a sample smaller than 1 microgram. That is a very exciting picture, but it must be tempered with a realization of the limitations of the method. In the first place, only molecules possessing permanent dipole moments will have pure rotational spectra. This immediately eliminates many desirable applications. Furthermore, many compounds of high molecular weight cannot be observed because of low vapor pressures or very low absorptions resulting from their large diameters. The use of wave guides with metal surfaces also introduces difficulties due to corrosion and to the adsorption of the material on the wall surface.

Microwave spectroscopy is particularly suited to the special analytical problem of determining relative abundances of isotopic species. With a change of mass of one of the constituent atoms there is often a large change in observed rotational frequencies. Because the individual lines may be correlated with molecules containing the individual isotopic species, there results an excellent technique for determining the abundance of each. It is probable that this method may supplant the use of the mass spectrometer for this purpose in many cases.

MASS SPECTROMETRY

In the mass spectrometer, a completely different phenomenon from those discussed above is utilized. In it the material under investigation is subjected to electron bombardment in the gas phase. As the kinetic energies of the electrons are larger than the sum of individual bond and ionization energies, the molecules are disintegrated into ionized fragments by impact. By a system of electrodes the ions are formed into a beam which then passes through a magnetic lens. This accomplishes a separation of the ionic species according to mass. This allows a mass spectrum, a plot relating the number of ions as a function of mass, to be obtained. Unlike compounds provide different mass spectra, and the differences in the spectra provide the basis of the analytical applications. It is probable that in no other tool of molecular spectroscopy are so many diverse devices of modern technology utilized. As a number of review articles (*18, 38, 64*) have adequately discussed the details of theory, construction, and operation of this instrument, no account of them is given here. An outline of the over-all processes may be seen in the flow diagram of Figure 16.

The ion current obtained for a particular mass is linearly dependent upon the partial pressures of all compounds present in the sample which may contribute to it. This makes it possible to obtain data for a set of ion peaks and to set up linear algebraic equations of the type given in Equation 3 relating the ion currents to the concentrations of the constituent compounds. The coefficients, which give the dependence of the ion currents on the concentrations of the individual compounds, are obtained by examination of the latter in pure state. When these are obtainable, the equations may be solved, thus yielding the analysis.

The gas is admitted to the ionization region where the pressure is of the order of 10^{-5} mm. of mercury through a leak from a chamber wherein the pressure is of the order of 0.3 mm. of mercury. In the ionization region the material must be in the gas phase. This places a limitation on the use of the device for the less volatile materials. Although it is possible to place a sample in a furnace within the ion source in order to produce a gas that may be ionized, this is a very special procedure and requires an instrument modified for the purpose. In this way, Hickam (*36*) has detected the impurities in copper with a sensitivity of 1 p.p.m. with 2-mg. samples.

The mass spectrometers that are used for extensive analytical work are equipped with automatic recording equipment. This suitably varies the electric or magnetic field so that the material is "scanned"—i.e., all the ionic species are successively brought to the detector, so that the mass spectrum, a plot of ion current as a function of mass, is automatically obtained. In view of the complex nature of some of the gas samples examined, this is done in a very short time. Complete sample time in the instrument including introduction, scanning, and removal may vary from 10 to 20 minutes. This rapidity of sample processing has been a most important factor in establishing this instrument in its present eminent place. It allows a mass spectrometer group using one instrument on one or several shifts per day to analyze several hundred gas samples per week.

The ionizing electrons are given an energy sufficient to ionize all types of molecules. Hence, if a compound is present in the sample in adequate concentration, data for it will be included in the recorded spectrum. If other materials contribute ions at the same masses, its presence may be evident only after calculation. However, multitudinous examples exist wherein the presence of a particular compound may be established from a single ion peak. In the case of polar materials, there is often difficulty in obtaining correct quantitative results due to adsorption of the material on the internal surfaces. However, indications are almost always recorded and there results a completeness of record, in the sense of acquiring data for all compounds present, that is hardly matched by any other gas analysis tool.

Gas Analysis. The analysis of gaseous mixtures is, in general procedure, very similar to the analysis by infrared absorption. The pure materials known to be present in the sample are scanned, masses chosen at which analytical data will be taken, and the corresponding calibration values determined. These are then applied to the data obtained for the mixtures through the system of linear equations mentioned and the individual concentrations are calculated. It is in gas analysis that the mass spectrometer has been economically the most important. It allows a considerably larger load of samples to be handled with a

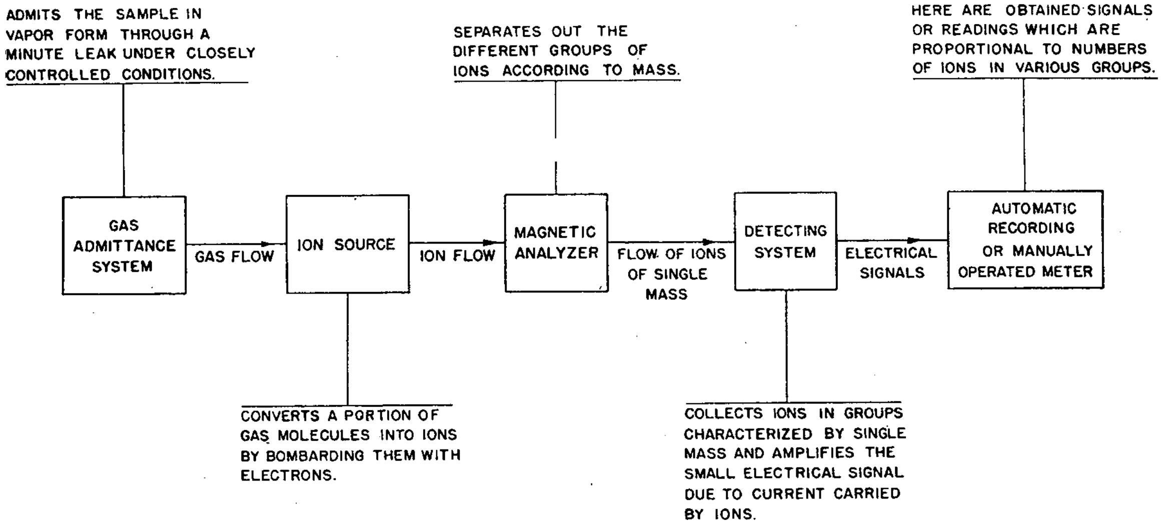


Figure 16. Block Diagram Demonstrating Steps and Process Utilized in a Recording Mass Spectrometer

Table XI. Comparison between Blended and Calculated Concentrations for Paraffin-Olefin Mixtures (*64*)

	C_1–C_6, %			C_2–C_5, %			C_3–C_5, %		
	Syn.	Calcd.	Diff.	Syn.	Calcd.	Diff.	Syn.	Calcd.	Diff.
H_2	3.0	3.2	+0.2	..	..	...	..	..	...
Methane	10.8	10.3	−0.5	..	..	...	..	..	...
Ethylene	6.0	5.9	−0.1	..	..	...	..	..	...
Ethane	6.0	5.8	−0.2	..	..	...	..	..	...
Propene	11.1	11.1	0.0	12.5	13.1	+0.6	13.2	13.4	+0.2
Propane	19.2	19.5	+0.3	24.8	24.9	+0.1	25.9	26.4	+0.5
Isobutane	20.1	20.0	−0.1	25.0	25.0	0.0	25.6	25.6	0.0
Isobutene	2.8	1.6	−1.2	3.8	3.6	−0.2	4.1	3.0	−1.1
n-Butene	2.9	4.1	+1.2	5.4	5.3	−0.1	5.4	6.5	+1.1
n-Butane	5.0	5.2	+0.2	7.5	7.7	+0.2	7.6	7.8	+0.2
Isopentane	10.1	10.2	+0.1	6.4	6.4	0.0	6.7	6.5	−0.2
Pentenes	3.0	3.1	+0.1	8.2	7.5	−0.7	4.9	4.6	−0.3
n-Pentene	..	..	...	6.4	6.5	+0.1	6.6	6.2	−0.4

smaller personnel than previously and the analyses are usually more complete and accurate. The petroleum industry uses this device more extensively than others. An example of the type of gas sample for which it is particularly valuable is one containing C_5 and lighter paraffins and olefins. In addition, it may contain hydrogen, oxygen, nitrogen, and carbon dioxide. Such a sample may be completely processed with a total operator time of the order of 1.25 hours, including the calculations. In Table XI may be seen a comparison between blended and calculated compositions for several samples.

It is to be noticed that in Table XI the pentenes are lumped together and the poorest accuracy was for the butenes. This is due to the close similarities of the mass spectra of the butenes as a class and of the pentenes as a class. It is, therefore, oftentimes common practice to lump the butenes in routine samples as well as the pentenes.

There is more difficulty in the analysis of polar gas due to adsorption effects. Such effects occur for sulfur dioxide, hydrogen sulfide, water, methanol, etc. In a few cases there is difficulty in attaining high accuracy, because the material has a low ionization efficiency, coupled with the fact that other compounds contribute ions of the same mass. Carbon monoxide is such an example. At its parent mass of 28, ion current contributions are given by nitrogen and by all hydrocarbons above acetylene in molecular weight. At mass 16 methane interferes and at mass 12 all the hydrocarbons plus carbon dioxide interfere. At apparent mass 14, at which the doubly charged ion of mass 28 appears, contributions come from nitrogen and all the hydrocarbons. Improvement in accuracy in such a case can come only by increased precision of measurements. However, for the polar gases, great improvement is being made by conditioning the instrument for a sample, introducing the sample almost directly into ion source, and heating the inlet systems. A number of gas analysis applications are described below in connection with elemental and isotope distribution measurements. An interesting analysis of the volatile products of metabolism, wherein several oxygenated compounds were found, has recently been reported (*24*).

Liquid Analysis. Any liquid sample that is examined by the mass spectrometer must be vaporized before passing through the leak into the ionization chamber. This limits the application to liquids possessing a vapor pressure of the order of 0.2 mm. of mercury or higher at room temperature. Raising the temperature of the inlet system allows less volatile liquids to be examined, but is to be avoided as a general practice. The general procedure for multicomponent analysis—the scanning of pure compounds, selection of masses, determination of calibration values—follows the same lines as for gases. The introduction problem is considerably more difficult for liquids than for gases. If the materials have vapor pressures of the order of 1 cm., reasonably accurate results may be obtained by vaporizing a sample into a standard volume and measuring the pressure with a manometer. For the lower pressures, the accuracy drops off and it is desirable to vaporize totally a known volume or mass of material. This is very satisfactorily done by means of sintered-glass valves (*11, 58, 59*). In this technique a sintered-glass disk is covered with mercury. As the mercury cannot pass through the disk, a vacuum may be maintained on the opposite side. If a small-bore pipet is brought through the mercury and into contact with the sintered disk, the fluid contained therein may pass through the disk and be vaporized into the evacuated space. This makes it possible to inject known amounts of fluid simply and to avoid the absorption of the heavy molecular weight materials by the stopcock greases.

As in the case of gas analysis, the petroleum industry probably makes the most extensive application of the mass spectrometer to liquid analysis problems. Surprising accuracy is attainable for hydrocarbon analysis, and the technique adds a tool of great power in the detailed analyses of mixtures in the gasoline range. In working with oxygenated and other polar compounds, the difficulties may, of course, be even more serious than for the polar gases, owing to the higher molecular weights. However, very promising results are now being obtained on mixtures of such materials by the use of special introduction systems and techniques (*22*). An idea of both the scope and precision of the method for liquid hydrocarbon analyses may be obtained from Table XII, which gives a comparison between blended and calculated concentrations for a mixture of C_7 paraffins. Considered in terms of the number of compounds handled, the fact that they are all isomers, and the relative newness of the method, the results are rather astonishing.

Table XII. Comparison between Blended and Calculated Composition of a Mixture of C_7 Paraffins (*11*)

Component	Known Composition		Determined Composition		Mean Difference
			Mole per cent		
2,2-Dimethylpentane	3.5	5.7	5.8	5.7	0.1
2,2,3-Trimethylpentane	2.2				
2,4-Dimethylpentane	50.7		48.9	49.3	1.6
3,3-Dimethylpentane	1.9		1.9	1.8	0.1
2,3-Dimethylpentane	31.7		33.0	33.6	1.6
2-Methylhexane	1.7		1.3	0.9	0.6
3-Methylhexane	3.8		4.6	3.0	0.8
3-Ethylpentane	1.8		1.6	2.5	0.5
n-Heptane	1.3		1.6	2.0	0.5
2,2,4-Trimethylpentane	1.4		1.3	1.2	0.2
Total	100.0		100.0	100.0	

Elemental and Isotope Dilution Analyses. The availability of the enriched stable isotopes in recent years has given the research worker a new tool for the tagging of molecules through synthesis. What is perhaps less commonly known is that it has made possible new analytical techniques for some very difficult analyses. Rittenberg (*49*) has described his isotope dilution method as applied to the determination of amino acids in proteins. It has been found that the determination of a particular protein, by standard chemical means, depends upon its isolation in the pure state and in quantitative yield from the protein hydrolyzate. Unfortunately, these two requirements are mutually contradictory. In the isotope dilution method, only one requirement—namely, isolation in the pure state—need be met. In this method a small amount of the amino acid sought, which has been synthesized with an excess of the heavy isotope N^{15}, is added to the hydrolyzate. In all subsequent operations the added amino acid and the same amino acid originally in the hydrolyzate are chemically inseparable. A small sample is isolated and degraded and the N^{15}/N^{14} ratio is determined. This ratio, together with the ratio for the naturally occurring material, the ratio for the added amino acid, the weight of the added amino acid, and the weight of original hydrolyzate, may be used to calculate the amount of the amino acid in the latter.

Grosse and his associates (*32*) have utilized this method in some very promising elemental analysis for carbon, oxygen, and nitrogen in organic compounds. For the oxygen determinations they utilized material enriched in O^{18}, for carbon they used material enriched in C^{13}, and for nitrogen they used material enriched in N^{15}. Instead of isolating some of the same type of material as was added, they subjected the material to temperatures above red heat and examined the pyrolysis products. They were able to show that equilibrium distribution among the isotopes was achieved even in the absence of complete combustion. Thus, in the determination of oxygen, it was only necessary to obtain data for some of the carbon dioxide obtained. Examples of the agreement between known and observed concentrations of oxygen in several compounds may be seen in Table XIII.

A somewhat similar method for the analysis for nitrogen, but without the use of enriched isotopes, has been reported by the same group (*37*). In this procedure nitrogen was formed by copper oxide reduction of the sample with subsequent reduction of the oxides by metallic copper. The nitrogen was then determined relative to the concentration of neon which had been added as an internal standard. This method obviates the use of the rather expensive enriched isotopes. A quantitative recovery of the gases is not necessary, as the ratio of nitrogen to neon is constant. With this procedure they achieved satisfactory agreement with known materials, among which were such compounds as pyridine, quinoline, indole, carbazole, and nitrobenzene.

Table XIII. Determination of Oxygen by the Isotope Dilution Method (*32*)

Analysis No.	1	2	3	4	5
Substance analyzed	Formic acid		Acetic acid	1-Nitroethane	Ethyl ether
Observed % oxygen	70.8	69.8	53.4	40.3	19.8
Known % oxygen	69.5	69.5	53.3	42.6	21.6

ACKNOWLEDGMENT

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Determination of Organic Functionality by Electrical Measurements

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The various electrical analytical methods are reviewed with respect to their general applicability, advantages, and limitations when applied to the determination of organic functional groups. The functional groups are considered separately and the electrical methods applicable to each are discussed. The greatest utility is found in the determination of acids and bases (amines), carbonyl compounds, halides, cyclic compounds, nitro compounds, olefinic and polynuclear aromatic hydrocarbons, phenols, peroxides, and sulfur compounds. Electrical methods are frequently of great value, particularly in special cases where other methods are not suitable or adequate. However, these methods cannot be taken as the ultimate solution to organic analysis in general because of the many varied types of interferences and complications encountered. Polarographic methods are the most outstanding and useful of the methods based on electrical measurements because they can be made rapid, are frequently applicable when other methods fail, and are often capable of differentiating between individual compounds or groups. Potentiometric, conductometric, amperometric, high-frequency, and polarized electrode (dead-stop) titrations are useful in detecting end points when a suitable indicator is not known, when the solution is dark in color, or when high sensitivity is desired. When they are used in conjunction with a mercury cathode and controlled potential electrolysis apparatus, coulometric methods appear promising and open a new field of investigation. Magnetic susceptibility has thus far found no apparent usefulness in practical analysis.

DURING recent years, steadily increasing emphasis has been placed on the development and use of instrumental methods of analysis, including those based on the measurement of electrical current or potential. Although much of the work has been confined to aqueous solutions and to inorganic materials, the problem of determining functional groups in organic substances has been aided by successful use of certain electrometric methods. In general, such methods have been of most value when other methods were not applicable or when speed was a critical factor. Polarographic methods have been most useful because they are often selective. Successful use has also been made of potentiometric, conductometric, amperometric, high-frequency, and polarized electrode (dead-stop) titrations, especially when an indicator could not be used or when high sensitivity or precision was desired.

In many cases, the scope of the electrical methods overlaps that of the conventional chemical methods or the recent photometric approaches. Experience has made it apparent (1) that all the electrical methods available are not always the best or ultimate means of determining functional groups in certain organic materials, because of interferences and other complications, and (2) that they sometimes only augment other analytical measurements. This creates a problem in the practical task of deciding which is the most suitable approach for the particular problem at hand and which, if any, of the electrical methods seems promising. It is this question which the present paper deals with by reviewing, in a very general way, the various electrical methods available and briefly discussing their utility and limitations when applied to the determination of organic functional groups. Thus, no attempt is made to present a thorough review of the literature, but many references are given to procedures that serve to illustrate the particular usefulness of each type of electrical method considered.

The literature already contains a number of excellent surveys and publications dealing with specific electrical methods. Thus, Furman (*11*) has reviewed the applications of potentiometric titrations to both organic and inorganic analysis. A bibliography of organic polarography has recently been published by Wawzonek (*52*) and a review of amperometric titrations has been given by Laitinen (*22*). The principles of polarography have been covered in detail by Kolthoff and Lingane (*19*) and Müller (*35*), potentiometric analysis by Kolthoff and Furman (*17*) and Michaelis (*29*), and conductometric analysis by Britton (*4*) and Shedlovsky (*45*).

The first part of the present review deals with electrical analytical methods of interest, comparing them with respect to their scope, advantages, and limitations when applied to the determination of organic functional groups. In the second part, the more important functional groups are considered separately and the electrical methods applicable to each are discussed; however, no detailed procedures are given. Finally, the relative attractiveness of electrical methods in contrast to other approaches is discussed and the factors involved in the choice of the particular electrical method to be used are considered.

CHARACTERISTICS AND SCOPE OF USEFUL ELECTROMETRIC METHODS

Potentiometric Titrations. Potentiometric titrations depend upon the measurement of the potential difference between two electrodes immersed in the solution being titrated, utilizing a reference electrode whose potential remains constant and an indicating electrode whose potential is a function of the concentration of a reactant in the solution. The end point of a titration is generally marked by an inflection or break in the curve relating potential to volume of titrant.

Probably the widest use of this method in organic analysis is in the titration of acids and bases in dark colored samples or in materials that destroy the indicator; it is also useful in the analysis of mixtures of acids or bases when differentiation is possible on the basis of their ionization constants. The scope of acid-base titrations has been extended by the use of acidic and basic solvents. Although some work has been done (*27, 57*), the use of nonaqueous solvents for the titration of acids and bases is a promising field for study and there is a need for the development of a practical nonaqueous reference electrode.

Compounds that form a reversible oxidation-reduction couple give stable potentials with a platinum electrode and, generally, can be titrated with an appropriate oxidizing or reducing agent. Certain other compounds which are oxidized or reduced irreversibly can also be titrated. These irreversible reactions usually require an excess of the standard solution, which is then back-titrated. The oxidation of hydroxy acids is generally reproducible, but requires the use of a different empirical factor for each acid. The application to redox titrations is somewhat limited because relatively few organic compounds can be selectively reduced or oxidized.

Organic compounds that can be precipitated or complexed by a metal ion can be titrated potentiometrically, provided an indicating electrode is available. It is possible to differentiate between certain compounds because of the difference in solubility of the respective precipitates, which causes more than one break to appear in the titration curve. Unfortunately, only a few types of compounds can be determined in this manner.

Under standardized conditions, the pH or millivolt reading at the mid-point of the titration furnishes some qualitative information. Thus it sometimes is a measure of the ionization constant of a weak acid or base, or a measure of the oxidation potential of a compound that forms a reversible oxidation-reduction couple.

In general, potentiometric methods are slower than indicator methods because of the time required for equilibrium at each of the large number of titration points required. However, in case large numbers of similar samples are to be analyzed, it is possible to make certain short cuts, such as titrating to a given potential, using dual or automatic titration apparatus (*23*, *38*), etc. Under favorable conditions, potentiometric titrations give an accuracy and precision of well under 0.5%. When poor titration breaks are obtained the precision can be in the range of 1 to 5%.

Conductometric Titrations. Conductometric titrations depend upon the measurement of the conductivity of the solution being titrated under conditions that avoid polarization of the electrodes. The conductivity is plotted against the volume of titrant and the end point is denoted by a change in slope of the curve. Because the conductivity is proportional to ion concentrations, the curve is usually a straight line before the end point and a straight line of different slope after the end point. The end point is found by extrapolating the two straight lines to their point of intersection, ignoring the actual deviating points near the end point. Obviously some temperature control is needed.

Acids and bases too weak to yield good potentiometric end points can sometimes be successfully titrated conductometrically. Thus phenols (*4*) can be titrated in alcohol-water mixtures with aqueous sodium hydroxide. Because conductivity is dependent upon the mobility of an ion, it is possible to differentiate between certain weak acids or bases that cannot be resolved potentiometrically. Some interesting work has been done on the titration of organic acids and bases in anhydrous benzene and dioxane (*28*), normally considered nonionizing solvents. No practical use has been made of this, however. Organic anions which are precipitated by metal ions (*4*) can also be determined conductometrically—for example, tartrate ion can be titrated with standard lead nitrate solution.

The presence of salts in the solution to be titrated lowers the precision of the method, because the changes in conductivity become relatively small compared to the total conductivity. Appreciable quantities of salts generally mask the end point completely. The precision and accuracy of conductometric titrations vary with the nature of the sample, being anywhere from 0.1 to 5%. The future of conductometric application seems dependent upon development of cells and meters for convenient measurement of the conductivity in nonaqueous media and of direct-reading meters suitable for ordinary laboratory use. There is a need for a highly stable, sensitive apparatus for application to highly conducting solutions such as those containing appreciable concentrations of salts.

Polarography. Polarographic analysis involves the measurement of the electrical current flowing through a solution when a potential is impressed between a dropping mercury (polarized) microelectrode and a reference electrode of large area, which may be simply a mercury pool. Conditions are such that the current measured is due entirely to the rate of diffusion of the substance being determined from the bulk of the solution to the surface of the mercury drop where it is reduced or oxidized. The current is then proportional to the concentration of the substance, provided the potential is great enough to cause its oxidation or reduction but low enough to avoid extraneous reactions. Generally, the current is found from a polarogram or graph obtained by manual or automatic plotting of the current obtained while the potential is progressively changed. The current is usually related to concentration by means of a suitable calibration curve.

A large number of organic compounds have been found to be oxidized or reduced at the dropping mercury electrode (*34*, *52*). The principal groups which are polarographically active are quinones, hydroquinones, aldehydes, α,β-unsaturated ketones, nitro and nitroso compounds, disulfides, polynuclear hydrocarbons, conjugated diolefins, unsaturated halides, and peroxides.

Polarography has certain advantages which are not shared by other methods of analysis. It is highly sensitive, so that trace (microgram) quantities can be determined, and it can be made very rapid. It is a direct measurement and does not require a reaction with another substance, as in a titration. In some cases, it can be made specific for a given compound of a group and under favorable circumstances more than one component in a mixture can be determined in one operation.

Unfortunately, polarographic analysis of organic compounds in mixtures is not always as useful as might be assumed. Relatively large concentrations of a substance which reduces at a more positive potential than the compound being determined interfere by masking the desired polarographic wave. Many compounds which are not active themselves at the dropping mercury electrode have a serious effect on the behavior of compounds which are active. Acetone, for instance, is inactive, yet changes both the half-wave potential and the wave height of acetaldehyde so that a correction must be applied which varies with the acetone content. Such interferences are not apparent until actual tests are made with the compounds that are expected to be present. Thus, before a dependable analysis can be made, it is generally necessary to know under the conditions of the test the effects of every substance present in the sample. Polarographic analysis, although very sensitive, is inherently less precise than titration methods and, therefore, is most useful for the determination of minor constituents. Generally the precision is about 3%.

The pH of the solution is a critical factor in the polarographic analysis of organic compounds because the hydrogen ion (proton) is usually involved in the reduction or oxidation. Both the half-wave potential and the wave height may vary with the pH. Only a few tenths of a pH unit can cause a noticeable variation in the results and, therefore, it must be known that the capacity of the buffer employed is great enough to take care of any acid or base that may be in the sample. Generally, it is advisable to check the pH after the sample has been added to the buffer solution. Another important consideration is the proper choice of an indifferent supporting electrolyte which is compatible with the sample yet allows electrolysis to proceed without interference.

Future developments will probably come from a systematic study of all organic functional groups that reduce or oxidize directly or that yield definite derivatives which are polarographically active. This will probably hinge on development of new electrolytes.

Amperometric Titrations. Amperometric titrations are a modification of polarographic analysis involving measurement of a diffusion current during a titration at a fixed applied potential. Either a dropping mercury electrode or a rotating platinum electrode is employed, a plot is made of diffusion current against volume of titrant, and the end point is obtained by extrapolation of the two straight lines in the titration curve to their point of intersection. The graph obtained is similar in appearance and use to that obtained in a conductometric titration. The amperometric approach can be applied to practically any titration in which one of the reactants is reducible or oxidizable at a polarized microelectrode and either appears in or disappears from the solution at the end point. Precipitation, oxidation-reduction, addition, substitution, and coupling reactions can form the basis for amperometric titrations.

The amperometric method has certain advantages over polarographic analysis in cases where it is applicable. The temperature need not be accurately regulated, but should be constant during the titration. It is theoretically capable of greater precision than polarographic analysis because the volume of titrant used and the precise indication of the end point allow more significant figures to be obtained; however, other factors such as the equilibrium constant may prevent this advantage from being realized. Because the equivalence point is found by extrapolation from the two regions in which there is an appreciable excess of one reactant over the other, it is not necessary for the titration reaction to be 100% complete at the equivalence point. It is only necessary that equilibrium be attained after each addition of titrant. Certain interferences affecting the wave height in polarography might not interfere in amperometric titrations if the effect remains constant during the titration. The substance being determined does not necessarily have to be reducible or oxidizable at the microelectrode as long as the titrant is active. Thus mercaptans (thiols) may be determined by titration with silver ion, although they do not reduce at the electrode. The method is generally faster than potentiometric titration, as relatively few points are necessary to establish the end point. It is also more sensitive than potentiometric titration, when both are applicable, because more dilute titrants can be used and smaller concentrations can be determined.

As in polarography, relatively large concentrations of substances which reduce at more positive potentials (or oxidize at more negative potentials) than the substance whose diffusion current is being measured seriously lower the sensitivity of the determination because the changes in diffusion current during the titration are then relatively small compared to the total diffusion current. This is similar to the interference of salts in conductometric titrations. Amperometric methods do not generally have the same selectivity as polarographic methods, because the selectivity of the method depends upon that of the titrant rather than on differences in half-wave potentials. Thus, in some cases, the two methods supplement each other.

This method has been applied to organic analysis in relatively few cases, but it appears to be a very valuable technique with many possible applications for future development.

Polarized Electrode (Dead-Stop) Titrations. Titrations with polarized inert electrodes, commonly known as dead-stop titrations (*10*), are possible when one of the reactants is a depolarizing agent. A small voltage is impressed across two platinum electrodes in a solution and the electrodes become polarized, owing to the absence of an oxidizing agent, so that no current flows. The appearance at the end point of a depolarizing (oxidizing) substance such as bromine or iodine causes a sudden flow of current. It is also possible to operate in the opposite way, so that the current ceases at the end point.

The method is generally faster than potentiometric titration, although not as widely applicable. When applicable, it compares in speed with indicator titrations, may be used with dark samples, often gives better precision than indicators, and is useful when no indicator will work.

The future of dead-stop titrations is closely connected to that of redox potentiometric and amperometric titrations.

Coulometric Analysis. Coulometric analysis involves the measurement of the quantity of electricity used in bringing about a quantitative chemical change. This quantity of electricity can be measured in any one of several ways, the most common being a gas or metal coulometer.

Lingane (*25*) has described a method of coulometric analysis in which the compound is reduced at a mercury cathode at a controlled potential and the quantity of electricity is determined from the volume of hydrogen and oxygen released in a gas coulometer. The electrolysis is considered finished when the current has decreased to a value near that which was obtained before addition of the sample. In order to attain selectivity, the constant cathode potential is chosen after consideration of the polarographic characteristics of the compound. Application of the method to the organic field is largely undeveloped. It has been used to determine the number of electrons involved in the reduction of organic compounds (*24*, *37*) at the dropping mercury electrode, as it is capable of greater precision than the polarographic method. Presumably, coulometric methods can be applied to the determination of any compound reducible at a mercury cathode, but side reactions may complicate many otherwise useful applications. The method is attractive in principle because it does not require a titrant; yet it is precise and can be made selective in favorable cases by proper selection of the cathode potential. However, its real value in practical organic analysis has yet to be determined.

A different type of coulometric analysis developed by Sease, Niemann, and Swift (*43*) involves electrolytic generation of bromine, which then reacts with the compound being determined. The bromine is generated at constant current and the quantity of electricity used is found by multiplying the current by the time of electrolysis. The presence of an excess of bromine is detected by the passage of current between two platinum auxiliary electrodes which are depolarized by the bromine. The current flowing between the auxiliary electrodes is proportional to the amount of excess bromine which is determined by extrapolating back to zero current. Thus the method of end-point detection has aspects of both dead-stop and amperometric titrations. An automatic apparatus for this determination has been developed by Shaffer, Briglio, and Brockman (*44*), who control the generation of bromine by the potential of a platinum indicator electrode and measure the quantity of electricity by an electronic microcoulometer.

Future use of coulometric analysis will await development of simple convenient apparatus and general exploration of applications to organic analysis. In this regard, microanalytical applications appear promising.

High Frequency (Electrodeless) Titrations. A high frequency oscillator has recently been utilized by Jensen and Parrack (*15*) to perform electrometric titrations without immersing electrodes in the solution. The titration vessel is placed within a coil of the oscillator and the plate current of the oscillator is plotted against the volume of titrant. As in conductometric and amperometric titrations, a change in slope marks the end point of the titration.

The instrument responds to changes in conductance and dielectric constant of the solution; thus, it can be used in place of familiar conductometric titrations. It is said to be more sensitive than conductance apparatus, so that oxidation-reduction titrations can be carried out, as well as acid-base and precipitation titrations. The fact that the instrument responds to changes in dielectric constant makes it possible, at least theoretically, to titrate in completely nonconducting media. Benzoic acid in acetone has been titrated with sodium methylate by this method.

Many laboratories are having difficulty in building a stable instrument with the proper response characteristics for this type of titration and, as yet, no such instrument is available on the market. However, the method promises to supplement other approaches and may be an approach to titrations involving the nonprotonic acids and bases of Lewis.

Magnetic Susceptibility. The force exerted on a sample when it is placed in a nonhomogeneous magnetic field is a measure of its magnetic susceptibility. This measurement has been made the basis for a titration of oxyhemoglobin with sodium dithionate (*30*) in which the magnetic susceptibility was plotted against the volume of titrant. A sharp change in slope indicated the end point. Magnetic susceptibility measurements have also been used to calculate the concentration of free radicals in a solution. However, the method has no apparent usefulness in practical analysis.

FUNCTIONAL GROUPS DETERMINABLE BY ELECTROMETRIC METHODS

The various organic functional groups which can be determined electrometrically are discussed below with particular reference to the approaches that have been used for each. Coulometric analysis, high-frequency titrations, and magnetic susceptibility are not mentioned in this section because they are not widely used at present. Presumably, coulometric analysis could be used in place of polarography for many applications in which the desired constituent is a major one. Similarly, high frequency titrations could be used in place of conductometric titrations, but only if the occasion warrants it.

The general information pertinent to each group is given in the preceding section under the method being discussed. No attempt is made to treat each subject exhaustively, but only items illustrating the practical or unusually informative applications are covered. Mention is made of the promising or successful application of electrical methods, even though better chemical or photometric methods are available. This is done because of the unique applicability of electrical methods to dark samples, to samples that decompose the indicator, or to those that contain certain materials that interfere with the conventional approach. No details of procedure are given.

Carboxylic Acids (Esters). The most widely applicable electrical method for organic acids is potentiometric titration. The determination is not specific, but it can classify the acids into groups differing in degree of ionization. The titration solvent (*57*) is varied to meet the particular situation at hand. For instance, a mixture of benzene and isopropyl alcohol (*27*) has proved particularly useful for the titration of mixtures of strong and moderately weak acids. Acetic acid (*13*) may be used as the solvent for very weak bases and ethylenediamine (*33*) has recently been suggested for the titration of very weak acids. An important application is the potentiometric determination of acids in oils (*1*). Various dibasic and hydroxy acids may be oxidized with cerate, and the excess cerate determined potentiometrically with a reducing agent (*54*).

Acids may also be determined by conductometric analysis (*4*), which is capable of determining much weaker acids in water or alcohol-water mixtures than can be done by potentiometric means. However, this method is not very satisfactory for resolving two weak acids of different strengths, unless both the degree of dissociation and ionic mobility are different for the two acids being determined.

Although the carboxylic acid group does not normally respond to polarographic analysis, a double bond next to the acid group is reducible. The determination of maleic and fumaric acids in the presence of each other (*51*) is an important example of this. Lactones can also be determined polarographically (*52*) in certain cases.

After hydrolysis or saponification in the conventional manner most esters can be determined by analysis of the acid produced, using either conductometric or potentiometric methods. A specific application is the potentiometric determination of esters in oils (*2*).

Phenols. The electrometric methods for phenols are not specific, but depend upon properties that are shared with many other types of compounds. Because phenols are weakly acidic they can often be titrated conductometrically (*4*) with sodium hydroxide in alcohol-water mixtures. Potentiometric titration can be carried out in ethylenediamine (*33*), a basic solvent, with sodium aminoethylate, a very strong base, as the titrant. This latter method has the advantage of affording a means of determining phenols in the presence of carboxylic acids. Recently, an amperometric method for naphthols has been suggested, involving titration with diazonium salts (*9*). Because simple phenols and cresols are readily brominated, they can be titrated with bromide-bromate using a dead-stop end point. Hydroquinones can be titrated potentiometrically with an oxidizing agent (*12*).

Amines. Amines and other nitrogen bases more strongly basic than aniline can generally be titrated potentiometrically in aqueous or alcoholic solutions. For weaker organic bases, the potentiometric titration with perchloric acid in glacial acetic acid (*13, 36, 55*) is now a well established method. Even the differentiation of primary, secondary, and tertiary amines (*48, 49*) has been found practical. The primary and secondary amines are acetylated and the tertiary amine is titrated in glacial acetic acid; the primary amine is converted to the azomethine with salicylaldehyde, and the secondary and tertiary amines are titrated in isopropyl alcohol. Large amounts of water interfere with potentiometric titrations in acetic acid and, in such cases, very weak bases such as pyridine and aniline can be titrated conductometrically in water or alcohol-water solution. Amperometric methods are applicable in certain cases; typical examples are the determination of aromatic amines by coupling with a diazonium salt (*9*) and the determination of amidines by precipitation with sodium alizarin sulfonate (*7*).

Aldehydes. Polarography is the only direct electrical method for the determination of aldehydes. It is especially useful because it allows the determination of certain aldehydes in the presence of others when there is a difference in their respective half-wave potentials. Thus formaldehyde can be determined in the presence of other aldehydes (*53*) and acrolein can be determined in the presence of saturated aldehydes (*32*). Similarly, aliphatic and aromatic aldehydes can be determined in the presence of each other.

A potentiometric method for aldehydes has recently been proposed (*46*), involving titration of excess acid after bisulfite addition. The titration is carried to a predetermined pH value depending upon the particular aldehyde being determined.

Ketones. Unlike aldehydes, simple ketones do not reduce under polarographic conditions. However, in the presence of excess hydrazine, phenylhydrazine (*26*), or Girard's reagent (*56*), the resulting derivatives of most ketones reduce and are determinable. Aromatic ketones, quinones, α,β-unsaturated ketones, α-diketones, and β-diketones reduce directly (*52*). The Girard derivatives of cyclohexanones do not reduce (*56*), making possible a differentiation between cyclohexanones and cyclopentanones.

Potentiometric titrations are applicable in certain cases. Quinones, for instance, can easily be titrated potentiometrically with thiosulfate solution (*42*). A recent hydroxylamine hydrochloride method for traces of acetone (*14*) involves potentiometric titration of the released acid to a predetermined pH value, after the initial pH is adjusted to this same value. A very rapid determination of acetone (*6*) has been suggested, which involves simply measurement of the pH of the solution after addition of hydroxylamine hydrochloride and comparison with a calibration curve. However, empirical determinations of this nature have the drawback that the potentiometric measurements are sometimes difficult to reproduce in the medium used.

Alcohols. There is no electrometric method for alcohols, as such, although certain chemical methods employ electrometric detection of end points in cases where this would be an advantage over indicators. Thus, the acid produced in the acetyl chloride method can best be titrated potentiometrically if the sample is dark in color. In the Karl Fischer method for alcohols (*31*), the dead-stop end point is considerably sharper than the visual end point. Glycerol may be titrated potentiometrically with dichromate (*39*).

Certain types of alcohols can be converted to other compounds which are more easily determined. An excellent example is the determination of ethylene glycol and 1,2-propylene glycol (*50*) in mixtures of the two. Reaction of these glycols with periodic acid produces acetaldehyde and formaldehyde, each of which can then be determined polarographically in the presence of the other.

Halides. In general, organic halides are unreactive but certain reactive types can be determined polarographically (*52*). These include halogen atoms on a carbon atom α- to a carbonyl or phenyl group, positive halogens, halogens attached to a metal which is attached to a carbon atom, and aromatic iodides and dihalides. Ethylene dichloride and ethylene dibromide can be determined in the presence of each other.

Sulfur Groups. Electrometric methods are particularly valuable for the determination of certain sulfur groups. Mercaptans can be titrated with silver nitrate either potentiometrically (*47*) or amperometrically (*18*). Amperometric titration is the more rapid of the two, but potentiometric titration is more useful for petroleum products because free sulfur and hydrogen sulfide can be determined simultaneously. Disulfides do not precipitate with silver but can be reduced to mercaptans (*20*) by treatment with zinc and determined as above. Polarographically (*52*), mercaptans give an anodic wave whereas disulfides give a cathodic wave. If the mercaptan sulfur is on the α carbon of an acid, a catalytic hydrogen wave is obtained in ammoniacal cobalt solution. This has been utilized in a method for the determination of proteins and cystene in urine (*40*). Carbon disulfide (*3*) can be converted to the xanthate with sodium hydroxide and alcohol and then titrated with iodine to a potentiometric or dead-stop end point; carbonyl sulfide behaves similarly. Thiodiglycol may be titrated amperometrically with bromine (*43*).

Nitro and Nitroso Compounds. Polarography has proved practical for nitro and nitroso compounds (*52*). Reduction of nitro compounds with excess standard titanous solution and back-titration with ferric alum (*21*) can be carried out using a potentiometric end point.

Olefins. The common chemical method for olefins involves the addition of bromine or iodine. A modification of the chemical method employing a bromide-bromate titration at 0° to 5° C. has recently been introduced (*8*) in which the dead-stop end point is used. This method appears to be generally superior to visual methods in both speed and reproducibility; the titration can also be done potentiometrically and amperometrically (*16*). Polarography can be used for the determination of phenyl-substituted olefins and acetylenes.

Polynuclear Hydrocarbons. Many polynuclear compounds are reducible at the dropping mercury electrode and this represents the only electrical method available for their determination. An important application is the polarographic method for the determination of naphthalenes in petroleum fractions (*5*).

Peroxides. Chemical reduction methods for the determination of peroxides could, of course, be followed potentiometrically in the case of dark colored samples. Polarographic analysis may also be used (*41*).

GENERAL CONSIDERATIONS

After the techniques applicable to each type of functional group have been considered it remains to decide whether or not an electrical method should be used for a particular problem and, if so, which approach should be applied. The solution of this problem must not be influenced by the inherent attractiveness of instrumental methods or by personal enthusiasm. Thus, no matter how elegant an instrumental method may appear, there is no point in using it if a simple method works as well or as conveniently. For example, the carbonyl group should be determined by a chemical, visual-indicator method unless an instrument gives the result sooner, more precisely, or more specifically.

Certain drawbacks inherent in the use of instrumental methods must be outweighed by other factors before the use of these methods is justified. Important among these are: (1) necessary specialized training, experience, and careful supervision required for good reliable work; (2) relatively great cost of suitable apparatus, and the expensive maintenance generally involved; (3) competitive uses to which the apparatus may be put; (4) large amount of time necessary for working out proper conditions, studying interferences, and generally getting acclimated, especially for nonrepetitive analyses; and (5) chances of getting into trouble through apparently small factors that may have a large, unpredictable effect (dirty capillary in polarography). Many of these drawbacks, especially the question of interferences, are also a consideration in the application of any analytical method.

However, other considerations often make the use of instrumental methods practical and worth while. These include speed, precision, and selectivity. In addition, the measurement obtained often contains qualitative information which is useful in itself and which makes possible the determination of more than one component. Thus, for specific uses, electrical methods can be very valuable and expedient.

The decision regarding which of the applicable methods should be used can be guided by the following generalities. Potentiometric and dead-stop titrations are better known and more readily used than other electrical methods because they are essentially a substitute for indicators. Conductometric titrations constitute an extension of certain potentiometric applications by permitting special benefits. All require relatively inexpensive apparatus and are not complicated.

Polarographic analysis is becoming widely familiar but still requires considerable knowledge and experience in interpretation of results, as well as advance information about the nature and composition of the sample. However, it is chosen in many cases because of its inherent speed, sensitivity, selectivity, and applicability to small amounts of sample. The related amperometric titrations are generally used when greater precision is required and when a suitable titrant is available.

The very fact that the measurements obtained by certain methods are complicated enough to require interpretation and study often makes them valuable because these complications can be resolved and special applications can be deduced. Thus the appearance of a seemingly anomalous polarographic wave or potentiometric plateau may indicate the presence of an otherwise unsuspected constituent and hence may provide a means of correctly calculating the constituent originally sought, which would otherwise have been in error.

Finally, the choice of the analytical method to be used is not always a simple one and considerable deliberation is necessary to arrive at a wise choice. The only exceptions are cases where there is no choice or where, for some reason, practical considerations are not important.

ACKNOWLEDGMENT

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Analysis of Cellulose Derivatives

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Cellulose derivatives, because of their high polymeric, carbohydrate structure, require modifications of conventional methods of organic analysis. Conditions of temperature, time, and reagent concentration must be kept within prescribed limits in order to avoid interfering side reactions and obtain accurate results. The acetyl content of cellulose acetate is ordinarily determined by saponification. Heterogeneous methods (saponification in suspension) are generally used, but methods based on saponification in solution have several advantages and are practical when the solubility behavior of the sample is known. Mixed esters of cellulose may be analyzed by the partition method, by isolating one acid by an extraction method, or by determining characteristic groups, such as halogen, alkoxyl carboxyl, or hydroxyl, in addition to total acyl content. Cellulose nitrate is analyzed by converting the nitrate group to nitric oxide and measuring the latter volumetrically. Methyl and ethyl ethers of cellulose are analyzed by modern versions of the Zeisel method and carboxymethylcellulose by titration of its carboxyl groups. Salts of the latter derivative may be analyzed by the methods of inorganic analysis after careful removal of organic matter. Precision is readily measured, but accuracy is difficult to establish because samples of known composition are not available. Complete analyses of some representative samples give an indication of the over-all accuracy of the methods employed.

THE analysis of cellulose derivatives presents a variety of problems. Variations must be made when the principles ordinarily used with simple organic compounds are applied, in order to avoid side reactions, and consideration must be given to the high polymeric nature of the samples. Conditions must be chosen to give quantitative results, yet avoid high results due to formation of undesirable by-products. Samples must occasionally be dissolved, filtered, and reprecipitated to remove impurities and to improve uniformity and physical condition.

Establishment of accuracy is a problem because samples of known composition are not available. Completely substituted derivatives are difficult to prepare, and the conventional methods of purification (distillation, crystallization, etc.) are not applicable. The precision can, of course, be evaluated more easily.

A series of papers from these laboratories describes some of the methods used in this field and their historical backgrounds (*8, 10–14*). Experience has shown that each sample or type of sample presents its own problem of analysis, and the conditions employed must be varied to conform with the properties of the sample.

CELLULOSE ESTERS

The tests commonly used for characterizing cellulose organic acid esters are shown in Figure 1. A technical man concerned with a practical use of the sample is interested in the solubility, viscosity (under use conditions), certain critical physical properties, and the stability of the batch. A theoretical chemist is more interested in its exact composition and a viscosity which is at least an approximate measure of average chain length. The interrelationships between these properties are shown by connecting lines in the figure. The determination of molecular weight distribution relationships, average molecular weight or degree of polymerization, and the ratio of unesterified primary to secondary hydroxyls are research problems at present. When this information is readily obtainable, it will be of considerable interest and use. This paper is confined to methods for the determination of composition.

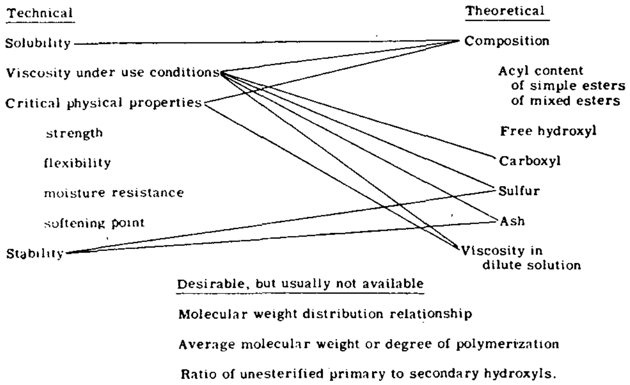


Figure 1. Tests for Characterizing a Cellulose Ester

Acyl Analysis by Saponification Methods. The variations in technique and limitations in conditions required are illustrated by the analysis of a simple ester, such as cellulose acetate, for its acyl (acetyl) content by saponification. In the older and more common heterogeneous methods in which the sample is saponified in suspension (*10* and literature cited therein), sufficient time must be allowed during the reaction, and particularly during the back-titration, for complete diffusion of reagents through the fibers of the sample. Conditions of alkalinity, time, and temperature must be vigorous enough to produce a complete saponification without any interfering side reactions, which are known to produce acid groups in carbohydrates. Figure 2 shows that at room temperature the alkali strength must be kept below 0.5 *N* in order to obtain an accurate result. Similarly, Figure 3 shows that the temperature must be kept below 40° C. When these limitations are met, the reaction time can be varied within reasonable limits.

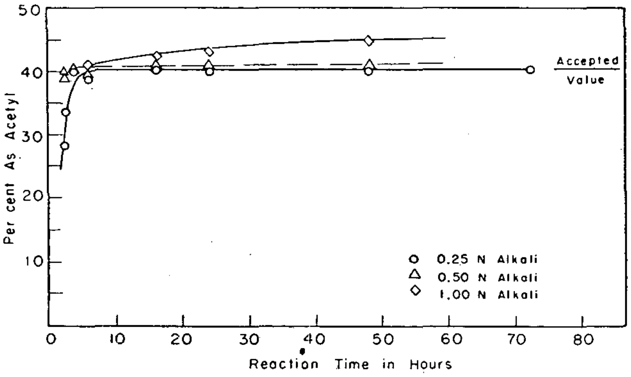


Figure 2. Effect of Alkali Concentration on Observed Acetyl Content of Sample of Cellulose Acetate

After using Eberstadt method

When an alkali normality of 1.0 and a temperature of 55° to 60° C. were used instead of the specified conditions, the observed acetyl content of a typical cellulose acetate was over 5% too high (*10*). About three fourths of this difference was shown to be due to nonvolatile acidity produced in the cellulose and about one fourth to volatile acids, mostly formic.

Limitations caused by the physical condition of the sample can be avoided and the elapsed time for an analysis shortened by use of methods based on saponification in solution (*4, 12*). These methods involve solution of the sample in a suitable solvent, followed by stepwise additions of alkali and water under conditions such that the ester remains in solution until saponification is nearly complete. The regenerated or only slightly esterified cellulose finally precipitates in a soft, finely divided form that does not interfere with the completion of the reaction or the back-titration. The solvents used and additions of alkali and solvent are varied, depending on the composition and solubility of the sample.

Acyl Analysis by Distillation Methods. It is often necessary to employ distillation methods for acetyl (or other volatile acyl) analysis when nonvolatile acid or other alkali-consuming groups are present. In addition to the classical acetyl methods involving solution in a strong mineral acid, distillation, and titration (*16*), several have been developed particularly for cellulose esters (*6, 10, 25*). Several difficulties, such as decomposition of the sample and possible retention of acid by the residue, are encountered, which are not met in ordinary organic analysis. In past work attempts have sometimes been made to obtain identical answers by distillation and saponification methods, but this may not be justified. More work is needed in this field on methods and on studies of precision and accuracy.

Higher Acyl Esters. The homologous series of esters of cellulose from formate to stearate covers a wide range of solubility and properties. The alcoholic alkali method (*10*) has been found to be applicable over this range, but good precision and accuracy are difficult to attain. The solution method, with modifications, is applicable up to about the caprate. Extraction methods have been used to isolate the higher acids, so they can be titrated directly.

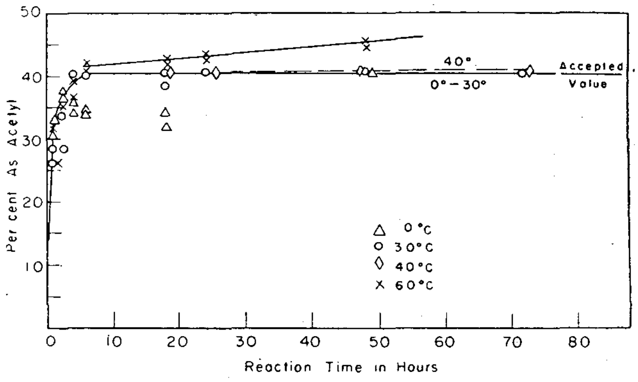


Figure 3. Effect of Temperature on Observed Acetyl Content of Sample of Cellulose Acetate

Using Eberstadt method with 0.25 *N* alkali

Hydroxyl. The hydroxyl content of cellulose esters may be measured by dissolving the sample in pyridine, acetylating with a solution of acetic anhydride in pyridine, decomposing the excess reagent with water, and titrating the acid present. The difference in titration between the sample and a blank is a measure of hydroxyl content (*12*).

This procedure is useful for the measurement of hydroxyl content and for estimation of other groups by difference, including application to the analysis of some mixed esters.

MIXED ESTERS

Partition Method. The partition method (*3, 13*), which is based on the work of Behrens (*5*) and Werkman (*23*), is generally applicable to esters of water-soluble acids and has proved to be satisfactory for everyday use. The steps in the analysis are:

1. Determination of total combined acids by a saponification method.

2. Saponification and isolation of combined acids by vacuum distillation or filtration.
3. Measurement of partition coefficients of both the mixture and its component acids between butyl acetate and water.
4. Calculation of the molar ratio of the acids.
5. Calculation of weight percentages from the molar ratio and the total acidity.

Table I shows partition coefficients of various acids (in 0.1 N solutions) between n-butyl acetate and water (*13*). The coefficients, k, show the fraction of acid remaining in the water layer after partitioning. They are equal to the volume of standard alkali required to titrate 25 ml. of extracted solution divided by the volume required to titrate 25 ml. of the unextracted solution. The differences between formic and acetic or between n-butyric and isobutyric acids are too small, but many other pairs or even sets of three (acetic, propionic, and butyric) can be analyzed satisfactorily. Other extractants and other ratios can be used.

Table I. Partition Coefficients of Various Acids[a]

Acid	k_1, 15 Ml. of Butyl Acetate, 30 Ml. of Water	k_2, 60 Ml. of Butyl Acetate, 30 Ml. of Water
Formic	0.885	0.660
Acetic	0.851	0.589
Propionic	0.592	0.263
n-Butyric	0.288	0.093
Isobutyric	0.280	0.090
n-Valeric	0.124	0.040
n-Caproic	0.036 (0.04 N solution used)	0.013
Crotonic	0.350	0.121
Methoxyacetic	0.920	0.752
Chloroacetic	0.532	0.233
Levulinic	0.869	0.624

[a] 0.1 N acid solutions used except as noted.

Extraction Method. Higher acids of the homologous fatty acid series such as stearic can be isolated from esters saponified by the alcoholic alkali procedure. The mixture is acidified with sulfuric acid and extracted with ether, and the ether solution is washed with water to remove the water-soluble acids. The ether solution of the higher acid is then evaporated to dryness, and the acid is dissolved in alcohol and water and titrated. The other acid present is calculated by difference from the saponification value.

The range of composition and properties of simple esters of this series and their various mixed esters is so great that variations of the methods are made for each particular case.

Characteristic-Group Methods. When one of the acids in a mixed ester has a characteristic group, such as carboxyl, halogen, methoxyl, etc., the analysis is comparatively simple. Carboxyl may be determined by direct titration; halogen, by adaptation of some of the standard methods; and methoxyl, as described below under cellulose ethers. The first acid is calculated directly from this information and the second is calculated by difference from total acyl content. Thus, cellulose acetate phthalates can be analyzed by carboxyl titration and apparent acetyl determination; cellulose acetate chloroacetate, from chlorine and apparent acetyl analyses; and cellulose acetate methoxyacetate, from methoxyl and apparent acetyl values.

Difference Method. By measuring the hydroxyl content as mentioned above, the acetyl or other acyl content in a simple ester may be calculated by difference. In the case of mixed esters the composition may be determined from hydroxyl and one other value, such as saponification value or the amount of some characteristic group present.

Physical Methods. The physical methods, including the mass spectrometer and the infrared spectrometer, have not been widely applied in this field, but they should be useful in special cases. Preliminary experiments (*21*) indicate that the mass spectrometer will analyze mixtures of n-butyric and isobutyric acids.

Nomographs. The above methods for analysis of cellulose esters ordinarily give results in terms of per cent of the characteristic group present. It is desirable from the standpoint of structural organic chemistry to convert this information into number of groups present per anhydroglucose unit. This can be done readily by means of nomographs such as Figure 4 (*8*).

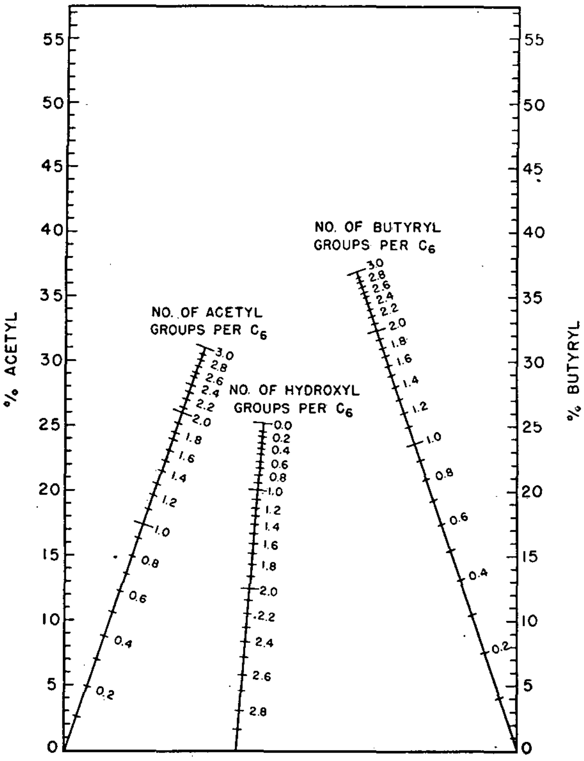


Figure 4. Nomograph for Converting Per Cent Acetyl and Butyryl in Cellulose Acetate Butyrate to Number of Acetyl and Butyryl Groups per Glucose Unit of Cellulose

A straight-edge placed across the acetyl value on the left scale and the butyryl value on the right scale shows at the intersections of the three center scales the number of acetyl, hydroxyl, and butyryl groups per anhydroglucose unit. In the case of cellulose acetates the stright-edge is placed across the acetyl value on the left and zero on the butyryl scale.

CELLULOSE NITRATE

The nitrogen content of cellulose nitrate is determined by the nitrometer method (*2*, *9*, *18*), which is based on the following reaction:

$$2HNO_3 + 3H_2SO_4 + 3Hg = 3HgSO_4 + 2\overline{NO} + 4H_2O$$

The nitric oxide is determined volumetrically. Nitric acid or inorganic nitrates can be measured directly as indicated. Cellulose nitrate is first dissolved in sulfuric acid and then transferred to the nitrometer. As in the case of the analysis of cellulose acetate, the time and conditions employed must be controlled or inaccurate results are obtained.

Nitrogen content can be correlated directly with average number of groups per anhydroglucose unit, as shown in Figure 5.

ETHERS

Methyl and Ethyl. The alkoxyl content of methyl and ethyl ethers of cellulose can be determined by modern versions (*20*) of the classical Zeisel (*26*) method. Fortunately, the constant-boiling hydriodic acid required for splitting alkoxyl groups produces enough degradation of the cellulose to make the analysis progress smoothly. The iodate formation and thiosulfate titration introduced into this procedure by Vieböck and Schwappach (*22*) are widely used. It has the advantage of a large titration for a small amount of alkoxyl (6 equivalents of thiosulfate per ether group). Figure 6 shows the number of ether groups per anhydroglucose unit corresponding to alkoxyl contents.

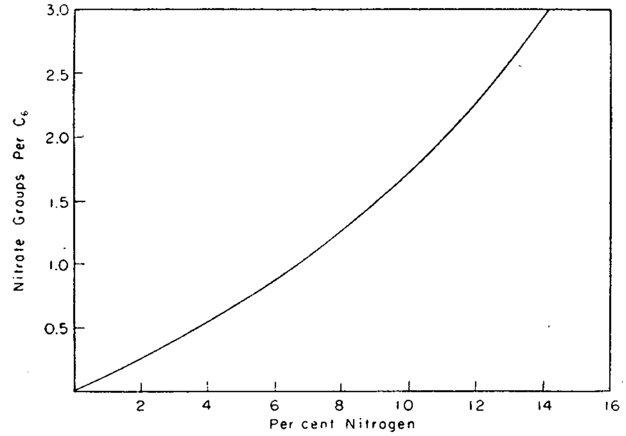


Figure 5. Relationship between Nitrogen Content and Degree of Nitration

Benzyl. Special methods proposed for the analysis of benzylcellulose (*15*) make use of a modified Zeisel procedure or conversion of the benzyl group to benzyl acetate by the action of acetic anhydride and sulfuric acid. The benzyl acetate is steam-distilled and determined by saponification. The carbon content of benzylcellulose is also a good measure of the degree of substitution and can be utilized conveniently when only an occasional sample is to be analyzed.

Carboxymethylcellulose and Its Salts. Several methods are available for the analysis of carboxymethyl cellulose and its various salts. The salts, if present, may be removed by treating with methanol acidified with hydrochloric or nitric acid, followed by removal of excess acid by washing with methanol-water solution and drying. The carboxyl content can then be determined by adding excess alkali and back-titrating, by a conductometric titration, or by liberating the acid as glycolic acid by refluxing with sulfuric acid solution and determining glycolic acid colorimetrically (*7*). The metal content of the sodium, potassium, aluminum, and zinc salts can be determined by dry ashing at a moderate temperature, treating with sulfuric acid and hydrogen peroxide, and analyzing the residues by methods of inorganic analysis (*19*)

PRECISION AND ACCURACY

The precision of analytical methods can be evaluated by means of control charts (*1*, *17*, *24*). Figure 7 shows acetyl values obtained on a typical cellulose acetate over a period of about 4 years by seven different operators. Individual values are charted, and the ranges of consecutive pairs of values were used to determine the control limits. The standard deviation of these 213 results is 0.12=, so the 3σ control limits are ±0.35% acetyl. The few points outside these limits are attributable to a new operator just learning the procedure. When this sample was analyzed 10 times in one set under the best possible conditions, a standard deviation of 0.03 was obtained. The long-time variation actually experienced was, therefore, four times as great.

Determination of the accuracy of methods for the analysis of cellulose derivatives is greatly complicated by the fact that the composition of these materials is not known definitely from their method of preparation. Accuracy can, therefore, be established only by complete analysis of representative samples combined with long experience with the methods in regular use. When samples of comparatively high degree of polymerization (greater than 200) are analyzed, the effect of the extra hydroxyls on the end groups is negligible and the sum of the acyl groups and unesterified hydroxyl groups should be 3 per anhydroglucose unit. Table II (*11*) shows results of analyses of some cellulose acetates, acetate propionates, and acetate butyrates. The agreement between the total observed groups per anhydroglucose unit and 3 may be taken as a verification of the over-all accuracy attained in this series of analyses. Sample W, which had a degree of polymerization of about 20, is an exception. The total number of groups observed was 3.111 as compared with a calculated number of 3.100 for this degree of polymerization.

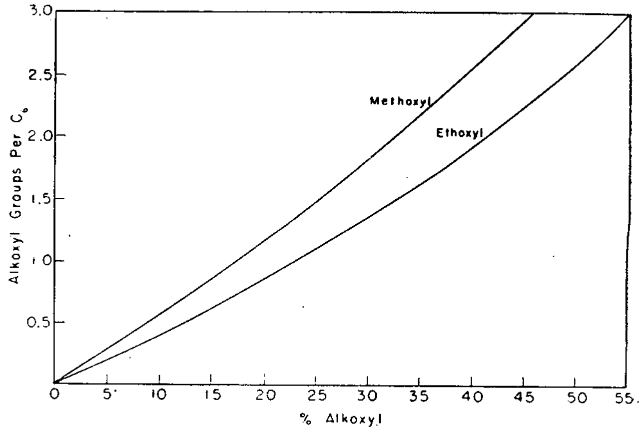
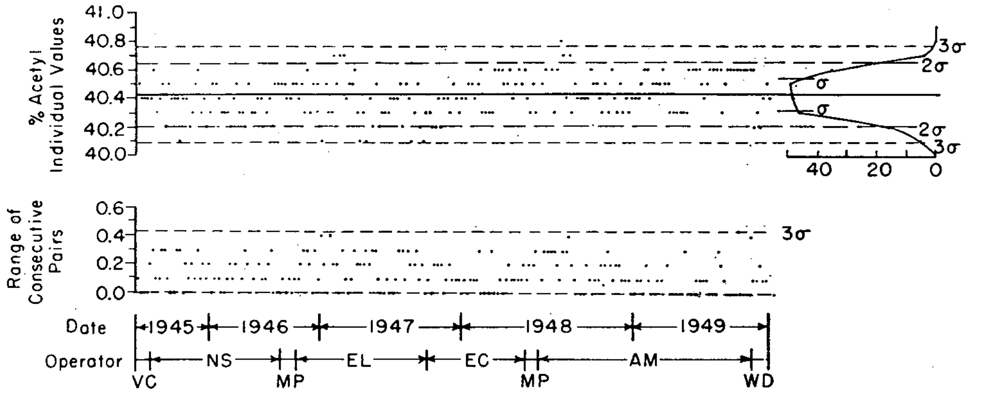


Figure 6. Alkoxyl Contents of Cellulose Ethers



Average of 213 Values Over 4 Years,
by 7 Operators 40.42% Acetyl
Standard Deviation 0.12-% Acetyl

Figure 7. Evaluation of Precision Using a Control Chart

Analysis of check sample of cellulose acetate by Eberstadt method

Table II. Analyses of Representative Cellulose Esters

Sample	Analysis % Acetyl	 % Propionyl	 % Hydroxyl	Groups per Anhydroglucose Unit of Cellulose Acetyls	 Propionyls	 Hydroxyls	 Total
			CELLULOSE ACETATES				
A	32.1	...	8.96	1.761	...	1.243	3.004
B	38.3	...	4.58	2.304	...	0.691	2.995
C	40.5	...	2.99	2.525	...	0.472	2.997
D	42.1	...	1.90	2.690		0.307	2.997
			CELLULOSE ACETATE PROPIONATES				
H	29.3	14.5	1.98	1.933	0.722	0.330	2.985
I	30.2	16.5	0.23	1.099	0.865	0.040	3.004
J	15.0	30.5	2.47	1.018	1.566	0.425	3.009
K	14.1	33.9	0.87	1.003	1.822	0.157	2.982
			CELLULOSE ACETATE BUTYRATES				
		% Butyryl			Butyryls		
S	30.9	16.2	0.86	2.160	0.686	0.152	2.998
T	29.5	17.5	1.16	2.060	0.740	0.205	3.005
U	17.6	28.3	3.55	1.209	1.177	0.616	3.002
V	2.6	49.8	2.54	0.203	2.301	0.495	2.999
W	11.3	43.6	0.09	0.926	2.167	0.018	3.111[a]

[a] Sample W had d.p. of about 20. In this case, therefore, number of extra hydroxyls on end groups is significant.

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Physical-Chemical Measurements and the Structure of the Penicillins

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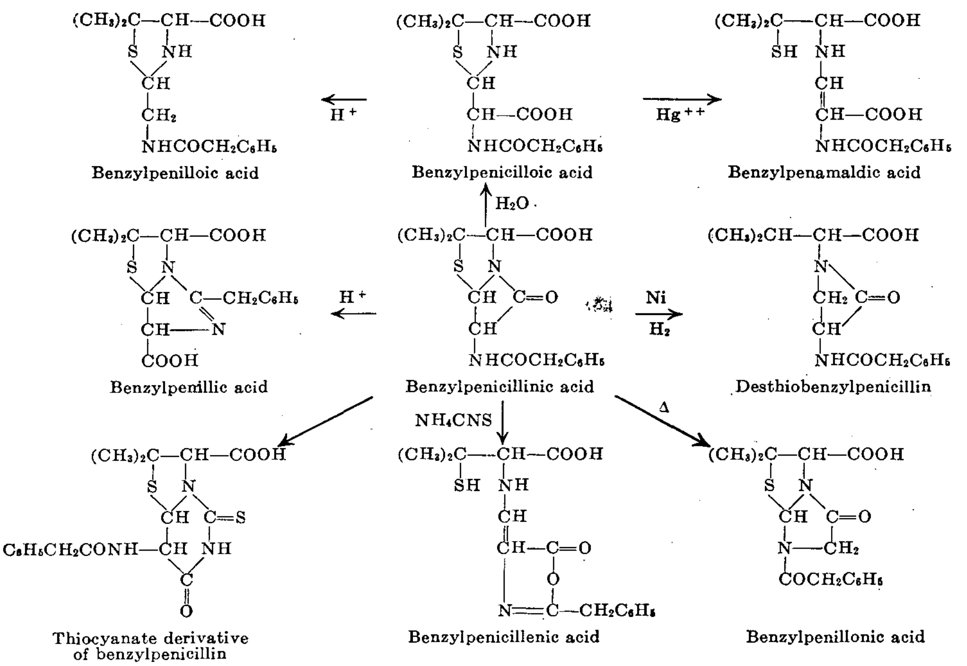
The application of physical-chemical methods to the elucidation of the basic structure of penicillin is reviewed. In particular, the unusually equivocal nature of the factors which entered the penicillin structure problem and the manner in which the physical-chemical approach permitted specific choices to be made are emphasized.

THE classical methods of organic chemistry proved entirely adequate for the elucidation of the structures of the deep-seated degradation products of the penicillins and consequently, although physical-chemical methods were used in connection with this work, the role of such methods was largely confirmatory in nature and in no real sense crucial. Thus, by December 1943, following the isolation in July 1943 of benzylpenicillin in pure crystalline form, all the simpler degradation products of that substance were known and their structures easily arrived at with assurance. Among the more important of these were D-penicillamine, phenylacetic acid, phenylacetamide, phenylacetylglycine, phenylacetylaminoacetaldehyde, benzylpenilloic acid, benzylpenicilloic acid, and its α-ester and α-benzylamido derivatives, as well as its decarboxylation product, carbon dioxide.

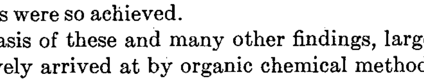
Further investigations of benzylpenicillin led to the isolation of a number of isomeric rearrangement products, such as benzylpenillic acid, benzylpenicillenic acid, and benzylpenillonic acid (Table I). These substances proved more difficult to analyze from a structural point of view and, in fact, physical-chemical methods played a more important role in the assignments of their now accepted structures. The ultraviolet absorption spectra of benzylpenillic acid and especially of benzylpenicillenic acid were of particular force in orientating thinking to the correct structures of these substances. In view of the fact, however, that this account of the role of physical-chemical methods in the penicillin structure problem must necessarily be restricted to its most important aspects, those interested in the more minor aspects of the general problem must be referred to a detailed account of the ultraviolet spectroscopy of benzylpenillic and of benzylpenicillenic acids (*1*). The generally accepted culmination of any struc-

Table I. Reactions of Penicillin



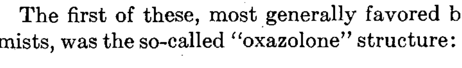
tural problem is the extension of the structural notions to a mode of synthesis; and syntheses of both benzylpenillic and benzylpenicillenic acids were so achieved.

On the basis of these and many other findings, largely though not exclusively arrived at by organic chemical methods, the majority of the penicillin investigations by early 1944 had settled upon two alternative structural formulations for the penicillin molecule. The first of these, most generally favored by the organic chemists, was the so-called "oxazolone" structure:



$$\begin{array}{llll} R\text{—}C\text{=}N\text{—}CH\text{—} & CH\text{—} & S\text{—} & C(CH_3)_2 \\ O\text{———}C\text{=}O & NH\text{——} & & CH\text{—}COOH \end{array}$$

The second was the so-called "lactam" structure:



$$\begin{array}{lll} R\text{—}CO\text{—}NH\text{—}CH\text{—}CH\text{—}S\text{—} & C(CH_3)_2 \\ \qquad\quad CO\text{—}N\text{———} & CH\text{—}COOH \end{array}$$

This formulation was acceptable to the physical chemists, because it satisfied the one condition which they regarded as essential in any proposed structure for the penicillins—namely, that the α carbonyl of the penaldate moiety be bonded to the thiazolidine ring nitrogen. At this early date, however, it was not possible to be dogmatic in one's acceptance of the β-lactam structure, inasmuch as related β-lactams were unknown. Naturally, in a problem involving so large a number of investigators, structures other than the above were suggested, but none of these ever attracted as wide attention and they were relatively quickly eliminated as additional properties of the penicillins became known.

During early 1944, considerable experimental effort, both degradative and synthetic, was devoted to conclusive proof of the "oxazolone" formulation. The synthetic efforts must be considered essentially negative or, at the very least, inconclusive [a very small biological activity of the order of 1 to 2 units or 0.001 mg. of penicillin, as finally demonstrated by the brilliant isolation work of du Vigneaud and his collaborators, was formed during the condensation of D-penicillamine and 2-benzyl-4-methoxymethylene-5(4)-oxazolone]. The additional degradative work, although it failed to result in the conclusive proof desired, contributed nothing to weaken the conviction of its supporters.

During this same period, those less convinced of the correctness of the "oxazolone" formulation, chiefly the physical chemists, were continuing to pursue a series of observations which had their origin and stimulus in the one discordant note in an otherwise essentially satisfactory and consistent structural picture.

Before elucidating the nature of this discordant note and the subsequent observations which followed it (all of which were chiefly of physical-chemical nature) and for reasons of greater emphasis and clarity as to the almost unique nature of the over-all penicillin structural problem, let us jump ahead in time and continue to follow the path of organic chemistry in order to see where it ultimately led from a structural point of view.

Late in 1944, as a result of the action of Raney nickel catalyst on sodium benzylpenicillinate, a crystalline desulfurized degradation product was obtained which on mild alkaline hydrolysis behaved like benzylpenicillin in the sense that it gave rise to D-α-desthiobenzylpenicilloic acid identical with the product obtained by the action of Raney nickel catalyst on α-methyl-D-α-benzylpenicilloate which could be obtained from benzylpenicillin by mild methanolysis. This benzylpenicillin desulfurization product was named desthiobenzylpenicillin and a study of its properties, both organic and physical-chemical, left little doubt that it was indeed a β-lactam. Moreover, because benzylpenicillin when treated under the same conditions, but in the absence of Raney nickel catalyst, could be recovered unchanged, it was inferred that intramolecular rearrangement had not taken place and that desthiopenicillin had the same structure, except for the loss of the sulfur atom, possessed by its progenitor penicillin.

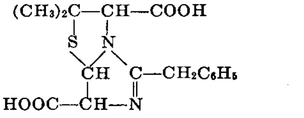
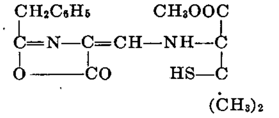
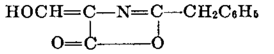
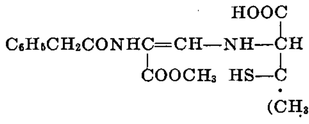
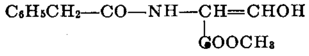
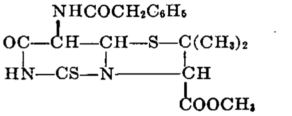
Thus, organic chemistry led to an equivocal situation and appeared to support both contending structures, the oxazolone structure in terms of the penicillenates and the lactam structure in terms of the desthiopenicillins! One could conclude from such experiences only that pencillin was too elusive a molecule to permit its structure to be determined by classical organic chemical methods, based as they are upon reactions under given conditions and the nature of the products so formed; and that, consequently, if its structure were to be obtained at all it would have to be accomplished in terms of the properties of the penicillin molecule itself. The properties of a molecule or, more particularly, its physical constants as related to structure are in the realm of physical chemistry, and consequently it would seem that only through its methods could the structure of penicillin be deduced with reasonable certainty and the log jam of equivocal findings broken. This, it appears to the author, has been achieved, and although some uncertainties still remain in the minds of some chemists, these would appear to be more philosophical than practical in nature.

Let us now consider the fact as obtained by essentially physical-chemical methods, restricting ourselves to the chief problem of the structure of the penicillin molecule and more specifically to an attempt to answer the question: "Does penicillin have a structure in which the carbonyl group (the α carboxyl of penicilloic acid) is bonded to the nitrogen atom in the 3 position of the thiazolidine ring?"

Table II. Dissociation Constants

Substance	pK_1		pK_2	
	Obsd.	Predicted	Obsd.	Predicted
5,5-Dimethylthiazolidine-4-carboxylic acid	1.58	...	5.98	...
Benzylpenilloic acid	1.5 (ca.)	1.4	4.75	4.8
α-Ethyl benzylpenicilloic acid	1.3 (ca.)	1.10	3.90	4.0
Benzylpenicillin	2.77	2.7–2.8	..	...
α-Ethyl-*N*-acetylbenzylpenicilloate	2.9	2.9	..	...
Desthiobenzylpenicillin	3.5	3.6	..	...
N-Acetylthiazolidine-4-carboxylic acid	2.96	...	..	...
Benzylpenillonic acid	3.0	3.0	..	...

Table III. Ultraviolet Absorption Spectra

Substance	Structure	Ultraviolet Absorption	
		$\lambda_{max.}$, A.	*EM*
Benzylpenillic acid		2350	4,600
Benzylpenicillenic acid		3200	28,000
2-Benzyl-4-hydroxy-methylene-5(4)-oxazolone		3000 2400	18,000 5,000
Benzylpenamaldic acid		2820	22,000
Methyl benzylpenaldate		2675	16,000
Thiocyanate derivative of methyl benzyl-penicillinate		2800 2370	16,000 12;000

ELECTROMETRIC BEHAVIOR OF PENICILLIN

A discordant note had been observed between the properties of penicillin and the oxazolone structure proposed for it: the acidity of penicillin. Early in the fall of 1943, a careful investigation of the potentiometric behavior of pure penicillin and its then known degradation products gave considerable support for the concept that penicillin is far too strong an acid to have the oxazolone structure (*2*). The structural conclusion as derived from the potentiometric studies of penicillin and related compounds took the following form. From a study of the thiazolidines, the penilloates, and the penicilloates, it was possible to develop an entirely consistent theory of the acid-base relationships of these compounds and to show that:

1. They are zwitterionic to the theoretically expected degree.
2. The inductive effects of a given substituent could be evaluated from simple types sufficiently accurately to predict the pK's to be expected of the structurally more complicated ones.
3. The electrostatic effect of a given polar substituent was shown, as expected, to decrease rapidly with distance from the ionizing center.

These simple rules, however, although they accounted very satisfactorily for the properties of such compounds, failed when applied to acetylthiazolidine, desthiobenzylpenicillin, benzylpenillonic acid, and benzylpenicillin. The reason for this failure would not seem hard to find, for the first three have no basic group and, therefore, cannot be treated zwitterionically. Many careful but unsuccessful attempts were made to detect a basic group in benzylpenicillin.

The behavior of benzylpenicillin in glacial acetic acid was particularly interesting. As is well known from the work of Conant and others, substances such as urea containing weakly basic groups are readily titrated in such a medium using perchloric acid. When applied to benzylpenicillin, no evidence of binding was observed until it decomposed as it does in glacial acetic acid, with the liberation of an imino group which could then be titrated quantitatively. One is forced to the conclusion then that penicillin has no basic group and that, in effect, it is like an *N*-acetylated thiazolidine. The effects of the sulfur atom in the thiazolidine nucleus on the pK_a of the carboxyl group can be estimated by comparison of the pK_a of *N*-acetylthiazolidine-4-carboxylic acid (pK_a 2.96) with that of *N*-acetylglycine (pK_a 3.6), and is 0.6 to 0.7 pK unit. In addition, the effect of an acetamino group can be obtained by the pK difference between *N*-acetylglycine and acetic acid (pK_a 4.8), and this amounts to 1.1 pK units. Inasmuch as the side chain amide group at the 2 position of the thiazolidine ring would not be expected to have any measurable effect (at most 0.2 pK unit) on the pK_a of the carboxyl group in penicillin (desthiobenzylpenicillin has a pK_a of 3.5, almost the same as for *N*-acetylglycine, pK_a 3.6), one concludes that the pK of the penicillins should be close to $4.8 - (0.7 + 1.1 + 0.2) = 2.8$. Benzylpenicillin was found to have a pK_a of 2.77.

These simple but forceful conclusions point directly to the β-lactam structure, which has all the characteristics required by the potentiometric properties. The salient pK_a data are presented in Table II.

ULTRAVIOLET SPECTROSCOPY OF PENICILLIN

The penicillins are substantially devoid of any characteristic absorption in the visible and ultraviolet regions of the radiation spectrum and consequently nothing of a positive nature concerning the structure of these substances could be learned through such direct measurements. Despite this, however, a surprising amount of information, of a structurally significant nature, was obtained through the use of ultraviolet spectroscopy under appropriate conditions. A significant number of the more important rearrangement and degradation products of the penicillins do possess characteristic ultraviolet absorption spectra, and quite apart from the value such spectra possessed in connection with the elucidation of the structures of these individual substances, they were of value in learning the nature (and speed) of the reactions that penicillin underwent under particular conditions.

Among the substances produced by penicillin having characteristic light absorptions are penillic acid, penicillenic acid, 2-benzyl-4-hydroxymethylene-5(4)-oxazolone, penamaldic acid, penaldic acid, and the thiocyanate derivative of benzylpenicillin, the last being a 5,6-dihydrothiouracil (Table III). Of the large number of interesting ultraviolet absorption studies made in connection with these compounds, only three are considered in detail here, as they had a direct bearing on the structure of penicillin. A study of the action of mercuric chloride in alcoholic solution upon benzylpenicillin and the α-methyl penicilloates, using ultraviolet spectroscopy as a means of following the speed and nature of the reaction, revealed that whereas the penicilloates were almost instantly converted in quantitative yield to the penamaldates, the *N*-acetylated penicilloates suffered no reaction whatever and benzylpenicillin reacted only very slowly. The slow reaction of benzylpenicillin was shown to be due to the fact

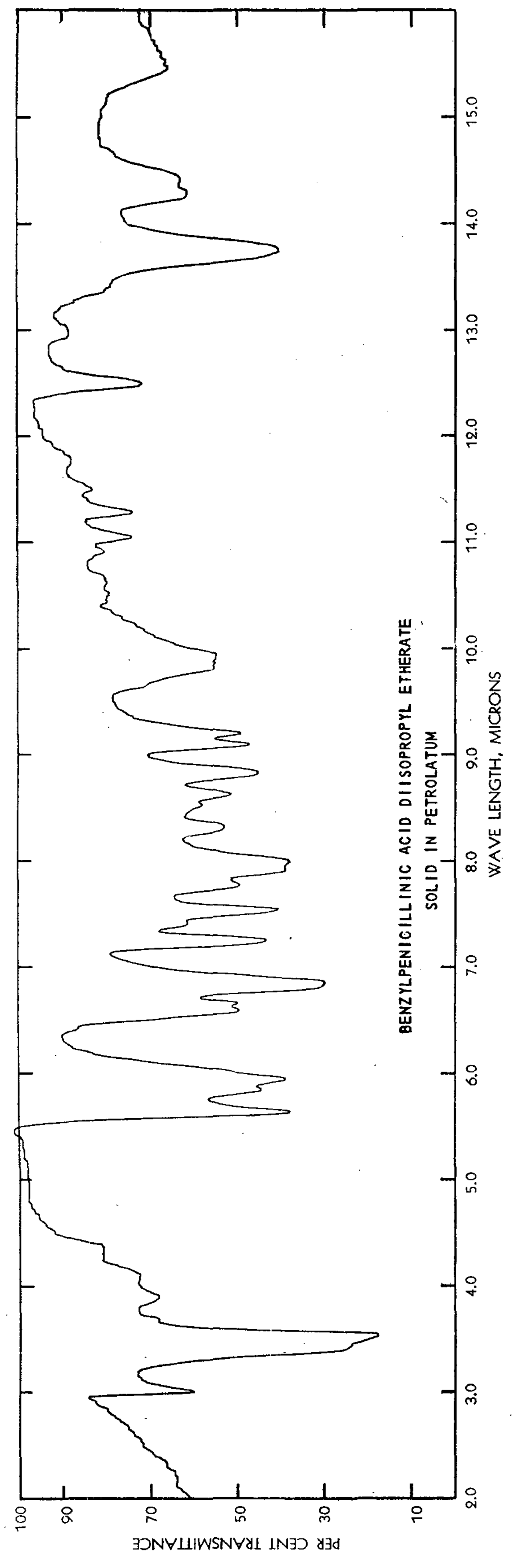


Figure 1. Spectra of Benzylpenicillinic Acid Diisopropyl Etherate in Petrolatum

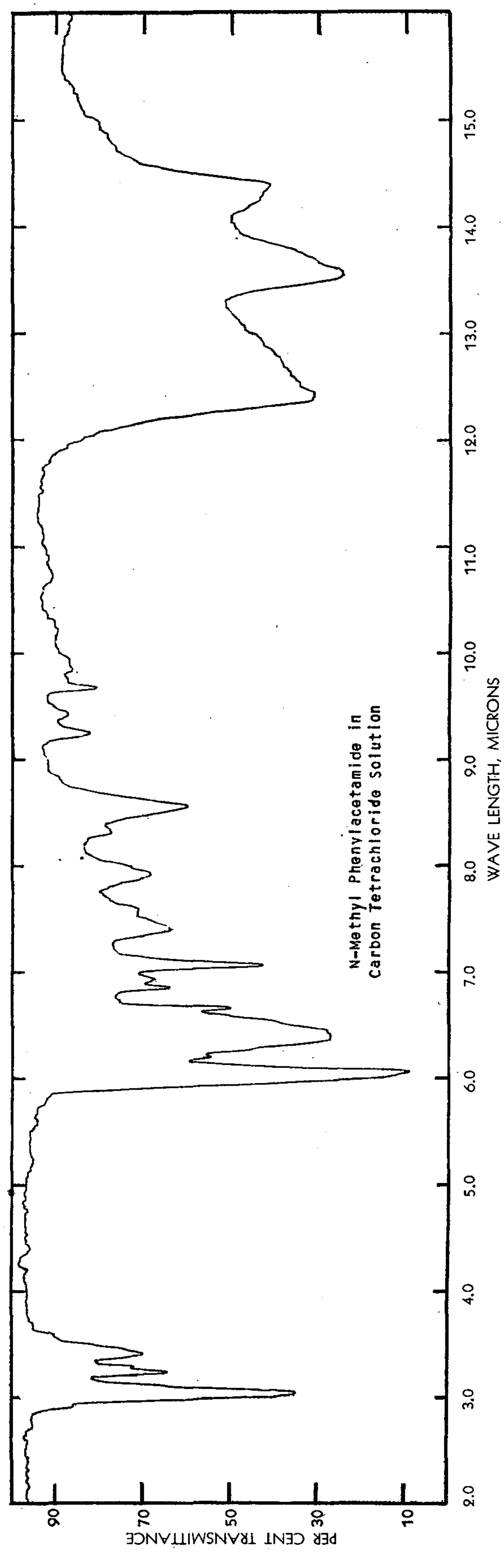


Figure 2. Spectra of *N*-Methyl Phenylacetamide in Carbon Tetrachloride Solution

that it suffered alcoholysis and thus formed α-methylbenzylpenicilloate, which then rapidly reacted with the mercuric chloride to form the corresponding penamaldate. This was demonstrated by the fact that if benzylpenicillin was allowed to stand for a given time in the alcohol alone, and then the mercuric chloride was added, the same amount of penamaldate absorption was produced at once (after the mercuric chloride addition) as was slowly produced in the same time in the presence of both reagents (alcohol and mercuric chloride). The obvious conclusion was drawn, therefore, that mercuric chloride does not react with benzylpenicillin any more than it does with the *N*-acetylated penicilloates and for the same reason—namely, that acetylation of the thiazolidine ring nitrogen stabilizes the 1,2 C—S bond to such a degree as to render it incapable of scission under the influence of mercuric ion. Such a behavior would be expected only if penicillin has a β-lactam structure and not if it has an oxazolone structure.

An ultraviolet absorption study of the dimethylacetal of benzylpenaldic acid under oxazolone-forming conditions revealed that instead of forming the corresponding dimethylacetal oxazolone, it always spontaneously lost a mole of methanol and quantitatively yielded instead 2-benzyl-4-methoxymethylene-5(4)-oxazolone, which could be identified in amount and character by its 3250 A. absorption band. This observation was taken as meaning that the oxazolone structure proposed for penicillin is inherently unstable and could have at best only transient existence. This conclusion was further supported by the ultraviolet-absorbing characteristics of the products obtained by means of the "azlactonizations" of the penicilloates or by means of the condensations of 2-benzyl-4-methoxymethylene-5(4)-oxazolone and D-penicillamine, both of which gave very high yields of penicillenic acid.

Ultraviolet absorption data proved of great value in the elucidation of the structure of the thiocyanate derivative of methyl benzylpenicillinate, which was unequivocally shown to be a 5,6-dihydrothiouracil. This proved to be an unexpected result, for the oxazolones had been known to produce thiohydantoins under such conditions. What would a β-lactam form? The question was finally answered when, by means of ultraviolet spectroscopy, it was shown that when the β-lactam of α-cyclohexylacetamido-β-cyclohexylaminopropionic acid (which could be synthesized) reacted with thiocyanate it also yielded a crystalline 5,6-dihydrothiouracil. Here again benzylpenicillin behaved like a β-lactam and not an oxazolone.

As a final illustration of the use of ultraviolet spectroscopy in connection with the structure of penicillin, let us consider its application to the elucidation of the structure of that key compound, desthiobenzylpenicillin. If this substance has a β-lactam structure, it should, on nitrosation, form an *N*-nitroso amide and not an *N*-nitroso amine. Through the use of a number of related model compounds, it was shown that the *N*-nitroso amides give products characterized by ultraviolet spectra having two bands of E_M 100 at 4250 and 4050 A. and a third band of E_M 6000 at 2450 A. On the other hand, the *N*-nitroso amines are characterized by one band of E_M 85 at 3600 A. and another of E_M 7000 at 2350 A. The crystalline nitroso derivative of methyl desthiobenzylpenicillin was found to show three absorption bands as follows: E_M 100 at 4250 A.; E_M 95 at 4050 A.; E_M 6800 at 2480 A. Obviously desthiopenicillin forms an *N*-nitroso amide and, therefore, has a free amidic side chain as required by its assigned structure.

The structural implications of these ultraviolet absorption studies, like those of the electrometric measurements, obviously support the β-lactam formulation for penicillin.

INFRARED SPECTROSCOPY OF PENICILLIN

The application of infrared spectroscopy to penicillin was not seriously undertaken until August 1944, and very soon thereafter it was apparent from the results obtained that only two formulations for the structure of the penicillins were possible, the oxazolone and the β-lactam, and that of these two the β-lactam seemed the most likely. Even at this late date the use of infrared spectroscopy in the elucidation of molecular structure or as a means of functional group analysis is predicated upon a semiempirical basis, at least for the more complicated molecules, and the infrared analyst is, therefore, very dependent upon the use of model compounds of known structure for a reasonable knowledge of the functional group frequencies characteristic of a particular class of molecules.

Because at the time of the launching of the infrared investigation of the penicillins nothing was known about the infrared-absorption characteristics of either the oxazolones or the β-lactams, it was necessary to obtain such data before any reliable analysis of the penicillin spectrum could be undertaken. The preparation of model oxazolones was a relatively simple matter, for many of such compounds were already known and in consequence the infrared spectra of a relatively large number of oxazolones were soon available. For the β-lactams, however, the spectroscopist found himself in the same position as the chemist, in the sense that they were almost unknown and no methods of preparation were, therefore, at hand. It became necessary to develop such methods, obtain reasonable proof of the β-lactam structure of the products, and then determine their physical and chemical properties (as model compounds) for comparative purposes. Thus developed one research problem within another, and it was not until about a year later that the necessary syntheses were accomplished and reasonably closely related β-lactams became available for spectroscopic studies.

In the interim the attention of infrared spectroscopists centered largely around two alternatives. Can the infrared spectrum of penicillin be accounted for in terms of an oxazolone or is the infrared spectrum of penicillin that of a monosubstituted amide? In connection with the latter part of this question, the infrared spectra of a truly large number of amides were recorded with many significant results.

The infrared spectrum of methyl benzylpenicillinate was found to be characterized by the following significant absorption bands: 3.0, 5.65, 5.72, 5.94, and 6.64μ, and for the sodium salt by 3.0, 5.65, 5.95, 6.20 and 6.60μ (Figure 1). The 3.0μ band in the linear stretching region of the infrared corresponds, of course, to the N—H frequency and is of no value in deciding between the two contending formulations. The 5.72μ band, which is absent in the sodium salt spectrum, and the 6.20μ band, which is absent in the methyl ester spectrum, originate from the carboxyl group, being the C=O frequency of the ester and the C=O frequency of the carboxylate ion, respectively. This leaves only the 5.65, 5.95, and 6.60μ bands to be accounted for. A survey of the infrared spectra of a large number of model 5(4)-oxazolones showed them to be characterized by two bands at 5.485μ for the C=O group and 5.97μ for the C=N group and by no other absorption bands down to 6.8μ. Moreover, a similar study of the simpler thiazolidines revealed that, as expected, they showed no absorption bands whatever between 5.5 and 6.8μ. Thus, on this basis, the 6.60μ band of benzylpenicillin is entirely unaccounted for and the 5.485μ band of the oxazolones is for some unaccountable reason shifted to 5.65μ in the penicillins. Again the oxazolone structure falls short of the mark.

Concurrent studies of the infrared absorption spectra of the monosubstituted amides, on the other hand, proved them to be very satisfactory models when it was found that they are characterized by two bands at 6.0 and 6.58μ (Figure 2). These bands correspond to the C=O stretching and N—H— deformation frequencies in such molecules. Deuterization of the simple monosubstituted amides, by exchange with deuterium oxide, effected a shift of their 6.60μ bands to 6.8μ, and precisely the same shift was observed for the 6.60μ band of benzylpenicillin after its deuterization. This not only strengthens belief in the amidic origin of these bands but supports the assignment of the 6.6μ band to the N—H deformation motion, the 6.60 → 6.80 shift being due to the N—H to N—D exchange. These observations left little doubt that the question concerning the presence of an amidic moiety in the penicillin structure had been answered affirmatively and that all but one, the 5.65μ band, of the critical infrared absorption bands had been accounted for.

Immediately the question arose: Is 5.65μ characteristic of the infrared absorptions of the C═O group in β-lactam rings when they are fused to thiazolidine rings? This proved difficult to answer for a long time. The first β-lactams available were sodium desthiobenzylpenicillinate and the β-lactam of α-cyclohexylacetamido-β-cyclohexylaminopropionic acid. These substances showed bands at 2.97, 5.80, 5.90, 6.31, and 6.60μ and 2.95, 5.77, 6.01, and 6.58μ, respectively. The amidic bands of these models at 5.90 and 6.60 and at 6.01 and 6.58μ were satisfactory, but the ring C═O bands at 5.80 and 5.77μ were discouragingly far from 5.65μ as required by the penicillins. It was pointed out, however, that these models contained no thiazolidine ring fused to the β-lactam ring and this fact might be the reason for their inadequacy. This indeed proved to be the case when, in the spring of 1945, three simple β-lactams with fused thiazolidine rings were synthesized by the Shell group. These substances showed infrared bands at 5.655, 5.625, and 5.63μ, and thus the last uncertainty concerning the correct interpretation of the infrared bands in the single- and double-bond regions of the penicillin spectra appeared removed. The infrared studies, like the electrometric and ultraviolet, pointed to the correctness of the β-lactam formulation.

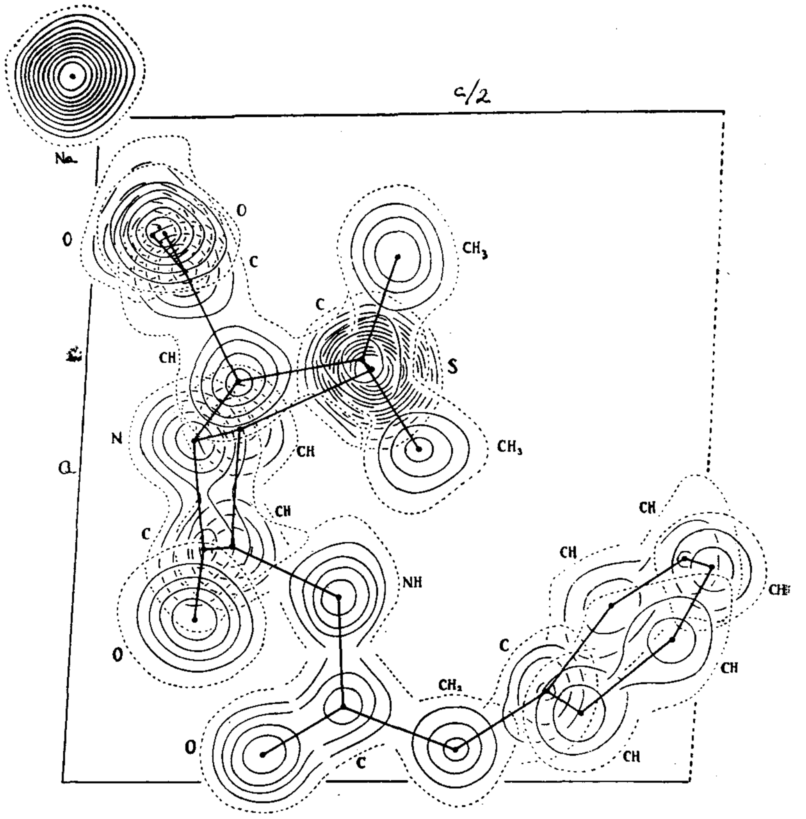


Figure 3. Electron Density Contour Map

Atomic positions of sodium benzylpenicillinate in b plane projection derived from second three-dimensional Fourier series

X-RAY DIFFRACTION SPECTROSCOPY OF PENICILLIN

These brilliant and monumental investigations, based upon the x-ray diffraction spectroscopy of the penicillins, provided a fitting culmination to the arduous assault upon their structure, and were carried out in England by D. Crowfoot and B. W. Rogers-Low in Oxford and by C. W. Bunn and A. Turner-Jones of the I.C.I. Research Laboratories. This work extended over approximately a 2-year period, during which the methods, both mathematical and physical, necessary for attacking so complicated a molecule as penicillin underwent steady processes of evolution and refinement, until at length a complete three-dimensional analysis of the structure was achieved which provided practically ocular proof of the correctness of the β-lactam formulation (Figure 3).

No attempt will be made to describe here, even in the briefest form, the details of this highly specialized and complicated work (*1*), although it might well be made the subject of a paper for some future symposium of this division. Only a brief summary of the results attained is considered in this paper.

Three crystalline salts of benzylpenicillin, potassium, rubidium, and sodium, were studied. The first two are isomorphous and belong to the monoclinic class of space group $P2_1$, having two molecules per unit cell. The third is orthorhombic, of space group $P2_12_12_1$ with four molecules per unit cell. In each instance electron density maps, projected upon the *a*, *b*, and *c* planes as well as complete three-dimensional Fourier syntheses, were computed, all of which resulted in the same atomic arrangements and the same atomic distances between atoms. This is a particularly notable achievement and contributes considerable support to the conclusion that the structure arrived at is the correct one, as each type of space group requires a different detailed method of treatment. Moreover, the bond lengths found for known parts of the molecule, such as the benzene ring, are in good accord with established values. As the illustration shows, all the atomic positions are individually resolved in the three-dimensional electron density maps of both the sodium and potassium salts, which would not be the case if false atomic coordinates had been used. False coordinates were found to give blurred electron density patterns. The estimated accuracy of the atomic distances is believed to be between 0.15 and 0.25 A., which is more than sufficient to place the atoms; indeed, 0.5 A. would have sufficed. These electron density maps leave no room for doubt about the existence of a β-lactam ring and an amidic side chain in the crystalline benzylpenicillin salts.

Naturally, a careful attempt was made to apply the same methods of treatment to the oxazolone formulation and, indeed, this was the first structure to be considered by the x-ray workers. They found, however, that much poorer electron density maps were obtained and that the deviations between the observed and calculated structural factors were unsatisfactorily large, whereas those obtained using the β-lactam formulation were about as good as those obtained by previous x-ray workers—i.e., 10 to 20%.

Some interesting details concerning the structure of the penicillin molecule come out of these x-ray analyses and are worthy of mention. As required by the chemical studies of penicillin, which showed that penicillamine has the D configuration while the carbon atom between the nitrogen and sulfur atoms of the thiazolidine ring has an L configuration, the derived x-ray structure shows these carbon atoms with opposed configurations. The fused thiazolidine and β-lactam rings are cis to each other, and, in each, one atom is out of the plane of others. In the thiazolidine ring it is the carbon atom attached to the carboxyl group, its displacement probably being due to the attraction of the carboxyl group for the metal ion in the crystal lattice. In the β-lactam ring it is the oxygen atom that is puckered. The positions of the atoms in the amidic side chain are particularly well defined and show normal bond lengths and angles, these being the same as those found in the simple amides. The C—N and C=O groups in the amidic side chain show no double- and single-bond character, respectively, and indeed the electron density contours around the nitrogen atom show a slight elongation suggestive of the presence of a hydrogen atom. These facts would seem to preclude enolization, hydrogen bonding, or dipolarization of the amidic atom group. The β-lactam oxygen atom and the amide oxygen atoms are 3.6 A. apart, which is too much to allow of hydrogen bonding.

The carboxyl groups are planar. A most interesting layering of the penicillin molecules is found to occur in the crystals of both the sodium and potassium salts. The molecules in both are so orientated in space and curled upon themselves that all four oxygen atoms are in the coordination sphere of the metallic ion and these are arranged around a screw axis, forming a plane of polar groups in these crystals. Similarly, the nonpolar methyl groups and benzene rings find themselves on the opposite side of the molecule away from the polar groups and so form a plane of nonpolar groups in these crystals. These polar and nonpolar planes alternate throughout the crystalline lattices.

CONCLUSION

It seems hardly necessary to expound further upon the important role which physical chemistry played in the penicillin structure problem. The facts speak for themselves. To quote one author, "No opportunity should be lost to emphasize the large part which physical methods played in the solution of a problem which, in the minds of many of the active investigators in the program, might still be obscure but for their use. It has been aptly expressed that, from a purely chemical point of view, the elucidation of the structure of penicillin was a problem of relative improbabilities" (*1*).

ACKNOWLEDGMENT

The author wishes to express his sincere thanks to C. W. Bunn of Imperial Chemical Industries Limited and to Dorothy Crowfoot, University Museum, Oxford, for providing the picture used in Figure 3, and to Princeton University Press for permission to publish it.

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[End of Symposium on Organic Functionality]

Current Trends in Analytical Chemistry

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The most significant features in the development of analytical chemistry during the past quarter of a century are discussed to indicate not only the direction in which analytical chemistry is moving, but also the probable direction of future progress. The principal types of activity in analytical fields are defined and their interrelations are made manifest. Outstanding examples are cited as exemplifying the wide scope of contemporary analytical chemistry. The relation of the analytical chemist to the general field of chemical activity is discussed.

THE present paper is the result of a survey of what has happened in analytical chemistry in the past few years and an attempt to note down the characteristic trends which have emerged in analytical chemistry in the past quarter of a century and, more particularly, in the past ten years. These trends seem of importance because they indicate the probable direction for much of future analytical research and development. Although many of the items discussed are familiar, there may be revealed some new device, technique, or approach to analytical problems, which offers possibilities in the reader's own fields of work. There have been included in this discussion several specific examples illustrating techniques and instruments, in order, as Gilbert and Sullivan made Pooh Bah say, "to give artistic verisimilitude to an otherwise bald and unconvincing narrative."

One at least potentially important current factor in analytical chemistry which will not be adequately considered is the influence of the huge amount of analytical work done by the Manhattan District and its successor organizations, which, presumably, has resulted in great advances in analytical chemistry. The Manhattan District analytical material (*15*) that has so far been declassified does not, on the whole, seem to offer any startling advances over known micro or other analytical techniques. However, the really significant material has probably yet to be revealed. The technique of activation analysis seems potentially to be of the greatest importance for the development of qualitative and quantitative analysis. The analytical possibilities associated with nuclear chemistry as well as the analytical techniques of nuclear chemistry were summarized in a recent symposium on the subject (*34*).

Before discussing the current tendencies in analytical chemistry, one should consider what is meant by that term. Twenty or thirty years ago there was little difficulty in defining analytical chemistry. Analytical chemists were then fairly well restricted to the inorganic mineral type of analysis. The enormous advances that had been made in the analysis of organic materials as in the Pregl techniques were largely due to and in the hands of organic chemists. At the same time, advances in analytical chemistry were being made at a tremendous rate by the biochemists, but the analytical chemists in industry and in the universities had, unfortunately, but little part in it. Most research in analytical chemistry in our academic institutions was restricted to the fundamental techniques of gravimetric and titrimetric methods, although men like Furman, Kolthoff, Mellon, and Willard were beginning to expand the field of academic analytical research. It is exactly 21 years since Nitchie's noteworthy paper on quantitative emission spectrography prophetically began the first issue of the ANALYTICAL EDITION in January 1929 (*32*); with the coming of age of ANALYTICAL CHEMISTRY, it is interesting to note the growth that has occurred in analytical chemistry during the childhood, adolescence, and teenhood of the journal.

Today, it is difficult to define analytical chemistry precisely.

As a first approximation, the subject may be limited, as specified previously by the author (*12*), to all techniques and methods for obtaining information regarding the composition, identity, purity, and constitution of samples of matter in terms of the kind, quantity, and groupings of atoms and molecules, as well as the determination of those physical properties and behavior which can be correlated with these objectives. Thus, analytical chemistry includes not only means of securing knowledge of the elemental composition, qualitative and quantitative, of materials, but also a knowledge of the way the atoms are put together to form the molecular units as, for example, in the determination of organic functional groupings. Although analytical chemistry does not include the use of x-ray diffraction in determining the unit cell of quartz, it does include the use of x-ray diffraction to determine the presence and amount of quartz in a sample of a dust or in a rock specimen.

Analysis may then be considered to be the application of analytical chemistry to obtaining qualitative and quantitative information about the nature of matter at the individual limit of the atomic level or higher.

It is, perhaps, not too fanciful to draw a parallel between the status of analytical chemistry a quarter of a century ago and the well-known situation regarding the state of physics in 1890.

All know the familiar story of how in the early eighteen nineties physicists considered that physics was almost a closed book, a completed story, and the only work to be done was the refinement of measurements and the extension of values to a greater certainty in the number of significant figures. Then along came the discoveries of x-ray and radioactivity, and physics broke wide open. The more or less static state assumed by physicists to exist at the beginning of that decade vanished, apparently never to return.

Analytical chemistry was regarded in a somewhat similar fashion two or three decades ago. While analytical chemistry was not regarded as something essentially complete, because there would always be a necessity for new methods of analysis for different mixtures of elements as in new minerals, most chemists felt that fundamental work in analytical chemistry or techniques had not at all changed since the early and middle of the nineteenth century when analytical chemistry was necessary and flourishing in order to prepare the bases for advancement in other fields of science. Especially vehement in pointing out that analytical chemistry had reached a static state and that nothing could be expected of it were some of the organic and physical chemists. Those who were or became interested in analytical chemistry as recently as the early or middle thirties may recall the tendency on the part of workers in other fields of chemistry to indicate the backward state of analytical chemistry. There were then people who pointed out that analytical chemistry in most cases was essentially the application of physical principles to elucidating problems of separation and measurement. However, these voices were more honored in the disregard paid them than in any other manner. One wonders what is the present attitude of the nonanalytical chemists just mentioned toward analytical chemistry, in view of the fact that many of them are now engaged in analytical chemistry together with many physicists, electrical engineers, and sundry other groups of specialists who apparently are not being very much disturbed by being workers in the area of chemical analysis.

After the foregoing introduction, one can discuss the tendencies and some of the concrete evidence of these tendencies which are believed to be predominant in contemporary analytical chemistry. Obviously, the techniques and apparatus to be discussed do not comprehend all the important developments, nor do they, for that matter, necessarily include the most important innovations or developments. The material discussed was selected to be representative of significant trends.

DEVELOPMENT OF MICRO SCALE TECHNIQUES

An important aspect in the current development of analytical chemistry is the stress being put on small-scale techniques, on the so-called semimicro, micro, and ultramicromethods. The term "so-called" is used because in many cases it is difficult to formulate a precise definition of what is meant by a micro or semimicromethod. If one starts with a sample of 10 grams and then measures several milligrams or micrograms of desired constituent, is the method a macro or a micro one? However, in most cases it is not necessary to name methods except for the purely formal purpose of classification. By now, most chemists know, more or less, what is meant by a microorganic method. When the discussion concerns semimicroorganic methods, though, a bit of difficulty is encountered. Although everyone will acknowledge that utilization of a sample of 3 to 5 mg. is microorganic analysis, a semimicroorganic analysis may include the use of a sample of anything from 10 to 100 mg. of a pure compound. From the viewpoint of sample size, many of the methods that have been brought into use recently are micromethods, for the sample needed may be only a few milligrams.

There is no need to discuss as such the development and applicability of micro or small-scale techniques, because this story is well known and has been competently discussed by Benedetti-Pichler, Clarke, Hallett, Kirner, Niederl, and others (*17*, *45*). However, one pertinent observation should be made. There has been a healthy development in respect to micro and semimicroanalysis in the rejection of the attitude that if a laboratory were a microanalytical laboratory it could and would do only microanalysis on every sample, heterogeneous or plentiful as the sample might be. At present, all or most analytical chemists feel that the analytical method used is not as important as obtaining certain information and that in order to obtain that information one should use any technique which will give it, including, as Beverly L. Clarke has suggested (*7*), medieval history, if the latter will help us. While Clarke's allusion to medieval history might have been a flight into rhetoric, a knowledge of ancient history may be of value to the analytical chemist. For example, the data of the interruption and eventual destruction of the Carthaginian sea trade in 146 B.C. turned out to be important for the chemist who was determining the composition of ancient coins and interpreting the results of the analysis, because at that period there occurs a profound change in the nature of the composition of the currency used, due to the cutting off of the supplies of tin imported from the British Isles by Carthage (*5*).

INSTRUMENTATION—MEASURING, RECORDING, AND CONTROLLING

One very impressive tendency of the past decade has been the tremendous advance made in the instrumentation of analytical techniques and methods for automatic operation, recording of data, and, finally, the controlling of chemical processes by the nature of the chemical data obtained.

The increasing importance of instrumentation in analytical chemistry will be amply indicated by the subsequent discussion. If further evidence of this trend is desired, reference need only be made to R. H. Müller's excellent monthly column in ANALYTICAL CHEMISTRY.

The movement over to instruments for analytical purposes, using the word "instruments" in a general or popular sense, is a very significant and important one. As Mellon and others have emphasized (*29*), analytical workers have for many years been using instruments in their daily work—precision measuring instruments such as the analytical balance and the buret. It is interesting to note that none of the so-called instrumental methods approach in precision of measurement the balance (1 part in 10^6) or the buret (1 part in 10^3 or 10^4). Generally, however, what are meant by instruments in analytical chemistry are devices for measuring the result of the interaction of energy, usually electromagnetic or electrical, with the sample as in absorption spectrophotometry or conductometry.

There are many reasons why instrumental methods are being more and more adapted and adopted for analytical measurements. These reasons are well known, but one point should be stressed. Although in many cases less experienced and less highly trained personnel can operate the instruments under normal conditions, highly skilled scientists are needed to maintain the instruments, develop methods involving their use, and interpret the results obtained. In addition, every new instrument, developed to solve or aid a given situation, by its unforeseen versatility and applicability develops a host of demands for its services in solving problems and questions—problems and questions formulated, in many cases, through having a means of answering them.

Recently, the vice president in charge of research and development of a large petroleum organization said that every time a chemist wants to buy an expensive piece of equipment for analytical purposes, the chemist always stresses the savings in man-hours and expense to be secured through having the desired instrument, and points out that the instrument can be operated by a bright high school girl. As soon as the apparatus is received, however, he hastens to point out that he should have an experienced Ph.D. to operate it, so as to get the full benefit of the advantages of the instrument. So, the vice president continued, the number of highly trained people engaged in analysis and the money spent for analysis continue to increase. The reason for this situation is, of course, the increasing demand being made for analytical information of greater variety, obtainable in a minimum of time.

Good examples of the instrumentation of well-known analytical methods are seen in the conversion to automatic operation of the micro and macromethods of organic combustion analysis by Hallett, Royer, Clark, the Shell Development Company group, and others (*17*, *18*, *45*). Apparatus have been described for the determination of ash, carbon, hydrogen, nitrogen, halogen, sulfur, and other elements. In the petroleum field instrumentation has been recently described, among other purposes, for long-term analytical distillation and aniline point determination (*25*, *35*).

The epitome in recent development in instrumentation has perhaps been reached in the Quantometer and other automatic recording emission spectrographs such as those developed by Saunderson (*38*), Nahstoll (*31*), and Hasler (*21*). This type of apparatus is the analytical instrument demanded by all—an instrument into which the sample is fed, and from or on which the results immediately appear. Given a suitable sample, the necessary maintenance and, importantly, the very necessary calibration and adjustment, the Quantometer does fulfill the production man's dream. In a similar direction, the analytical balance is being revised to speed up and simplify weighing.

Parenthetically, the worker in analytical chemistry is one of the few creatures, a great part of whose endeavor is devoted to rendering themselves as superfluous as possible. This half-truth emphasizes that the instrumentation of analytical methods lessens the amount of repetitive manual routine work to be done and simultaneously increases the quantity and quality of research and development necessary.

A great deal of work on instrumentation has been concerned with the development of techniques and apparatus for the automatic analysis of tanks or flowing streams of material, supplemented by the recording of the analysis and, occasionally, control of the producing reaction or process. Such methods usually involve the measurement of some physical property. Among the physical properties readily measured for automatic analysis are density or specific gravity, refractive index, thermal or electrical conductance, radiant energy absorption, pH, heat of combustion, viscosity, and others, including combinations of these techniques with chemical treatment if helpful. The use of such techniques results in a considerable saving in man-hours and analysis time, and, moreover, permits the research worker or plant operator to get his analytical result in a minimum of time.

It is only fitting that at this point due credit for much of the development in current analytical instrumentation be given to those unsung men—the analytical chemists' helpers and, often, publicity agents—the instrument and apparatus dealers. Some of the readers may recall an amusing and interesting paper published in 1931 by G. Frederick Smith entitled "Applied Analytical Chemical Research Necessitates Promotional Advertising" (*39*). It would profit all analytical chemists to read this article, and, after having read it, no longer to hide their light under a bushel but to get out and proclaim to the world the value of analytical chemistry and the very fine work being done in it. The analyst should bear this in mind: After all the disrespect shown by so many organic chemists for the typical old-style analysts, how many organic chemists obtain in every reaction the 100% yield demanded of the gravimetric analyst for every determination that he does?

The instrument and apparatus dealers by their investigational work on instruments, and by their instrumentation of analytical techniques and apparatus, have done a great deal to aid the analytical chemist in his work. In addition, their bold advertisements, when brought to the attention of the vice president in charge of research and development, have indicated to him that analytical chemistry is no longer in many cases a matter of only beakers and test tubes, valuable as these are; analytical chemistry has taken unto itself optics and electronics. Unfortunately, somewhat exuberant advertising has in some cases convinced the vice president in charge of research and development that if the company were to buy, for example, a mass spectrometer, all analytical problems would be immediately solved. All that would then be necessary to determine the carbon in an alloy steel would be to send the sample to the laboratory where, after the pressing of suitable buttons and the pulling of suitable levers, lights would glow and gears would whir, and the right answer would immediately appear.

This bit of hyperbole leads to a topic with which all analytical chemists should be concerned. It has been advertised of many pieces of apparatus that any high school youngster can be taught to operate them; this is probably true. But, in order to set up an analytical procedure or to interpret the results obtained, not to mention the maintenance of the instrument, it is essential that people of the requisite skill and ability be obtained. Too often, it would seem, the analytical chemist in industry and elsewhere has not been able to get across the fact that all our gorgeous instruments, important and valuable as they are, are not in themselves analytical chemistry. All these instruments and tools are just tools in the hands of the people who have to use them. The value to be gotten from these tools is great but only in proportion to the basic analytical feeling of the men using them. The situation is strongly reminiscent of that in the well-known story of the office manager who complained of the poor quality of the work being done by the girls in the office. The stenographers and clerks finally convinced him that the cause of the poor work was the antiquated office machinery they had to use. So the office manager went out and purchased new dictaphones, typewriters, adding and tabulating machines, and the other necessary business machines. He eagerly awaited the high quality work which these machines should produce. When he saw the first batch of letters, his face fell and he said mournfully, "The new typewriters don't spell any better than the old ones did."

DETERMINATION OF COMPOUNDS AS SUCH

One of the most interesting phenomena, which can be currently witnessed in analytical chemistry, is the increasing emphasis on the determination of structural or organizational composition in addition to the determination of elementary composition. While the organic analytical chemist has for years been determining hydroxyl, alkoxyl, and other functional groups, the general analytical chemist, restricted as he has often been to straightforward inorganic gravimetric, titrimetric, or colorimetric methods of analysis, has been mostly concerned with determining the elements, although groupings like sulfate and phosphate have been measured and it is customary in mineral analysis to report the results in term of oxides in order to reach a 100% figure on summation. At the present time, there are available techniques like those of x-ray diffraction and petrographic microscopy which often enable us to determine the actual compounds present. In addition, techniques such as polarography and molecular spectroscopy often permit the determination of the types of organic groups present and yield considerable information regarding structure. For example, the Girard derivatives of ketosteroids containing the carbonyl group in a five-membered ring are polarographically reducible, while the Girard derivatives of the corresponding six-membered ring compounds are not reducible.

An increasing percentage of papers in the literature which describe new compounds, presents optical and crystal data on these compounds as obtained by the polarizing microscope and x-ray diffraction apparatus. Such data have been especially valuable in describing isomeric organic compounds such as the isomers of DDT and of hexachlorocyclohexane. As more data become available on the optical constants and x-ray patterns of inorganic and organic compounds, still greater attention will be paid to the determination of compounds as single units instead of as collections of separately determined parts. The systematic publication of such data in ANALYTICAL CHEMISTRY is a significant development.

It is reasonable to assume that in the future the analyst will actually be able to answer readily the specific questions involved in the analyses requested. Thus, when someone asks for the butyl acetate content of a liquid mixture, the report will state not the amount of saponification in terms of butyl acetate, as is most often done, but the butyl acetate present, actually determined by not too difficult a method. When the production metallurgist will, in the not too distant future, ask for carbon in steel, he will expect more than a figure for total carbon content or for graphite and nongraphite carbon. He will ask for, and will receive, the carbon analysis in terms of the forms in which the carbon is actually present in the steel and within the short time now required to determine total carbon. Similarly, correspondingly complete information would be available from the determination of sulfur in gasoline.

MORE EXTENSIVE UTILIZATION OF KNOWN TECHNIQUES

It is proper to consider next the more extensive utilization of known techniques which is in many ways the foundation of any scientific field of inquiry. In the past few years much more extensive use has been made of techniques which heretofore have been used to but a slight extent in analytical work. Probably the outstanding example of these blossoming techniques is in the enormous application that has been made of radiant energy absorption or absorption spectrophotometry in the nonvisual region during the past eight years. The necessities of the wartime programs for the production of high octane gasoline and of butadiene and styrene for synthetic rubber gave a very noticeable impetus to the use of infrared and ultraviolet absorption spectrophotometry.

Ten years ago anyone who desired to use infrared spectrophotometry for analytical use would have had to build his own instrument; this situation, naturally, did not encourage a widespread analytical utilization of the infrared. Now, some half a dozen companies are making and selling various types of infrared spectrophotometers—instruments for manual operation, automatic recording of the spectrum, automatic recording of the concentration by measurement of the absorption at a selected wave length, and automatic control of a process operation based on the measurement of concentration.

A good example of the momentum given to use of a given technique or principle by a properly instrumented apparatus embodying it is seen in the Beckman spectrophotometer for visible light and ultraviolet absorption spectrophotometry. It is both interesting and instructive to survey the analytical literature of the past decade and to note the many applications in ultraviolet absorption spectrophotometry due to this instrument.

In addition to the ultraviolet and infrared spectrophotometers, either commercially available or described in the literature, a considerable number of very interesting ultraviolet and infrared photometers and filter photometers, based on novel principles, have appeared.

For example, ultraviolet photometers have been made, which depended on the use of a photocell of relatively narrow response range in terms of wave length. Thus, a Westinghouse tantalum cathode photocell, which responds only to radiant energy in the range of 2000 to 3000 A., can serve to measure the absorption of radiant energy and the corresponding concentration of conjugated dienes, aromatics, aldehydes, and certain other absorbing substances. The experimental apparatus is simple in principle and often in practice: a source of ultraviolet radiant energy such as a hydrogen discharge tube, a sample cell, and a photocell with its accompanying energy response indicator. The use of line spectra as monochromatic sources is another field of vast opportunity for analytical spectrophotometry, especially in multicomponent analysis.

Although the mass spectrometer was originally devised and almost exclusively used as an analytical instrument, it did not come into use in the wider fields of standard analytical chemistry until the past few years when the needs of the high octane gasoline and synthetic rubber programs created a demand for analytical techniques to satisfy specific desires for information. While ten years ago, or even less, a man would have been suspect who dared asked where he could buy a mass spectrometer, today at least three companies in this country are competing for his business. The strict analytical applicability of the instrument has been enlarged from hydrocarbons to include oxygenated and nitrogen-containing organic compounds.

From the analytical standpoint it is significant to realize that the mass spectrometer is essentially a means of separation. Its primary function and principal purpose is to sort out particles of matter according to their ratio of mass to electrical charge. The measurement of the total amount of material falling at each locus of ratio is a relatively minor affair. This is seen in the preparation of isotopes where the mass spectrometer principle is used to effect separation without measurement.

All remember the flame tests which have been used in freshman and sophomore qualitative analysis courses for nearly a century in identifying the alkali and alkaline earth metal elements. The conversion of these familiar qualitative flame tests into a useful quantitative tool for many elements is a good illustration of how known methods are being instrumented and brought into use in analytical chemistry. By now, at least three models of the flame photometer are commercially available. In this instrument, the sample solution is atomized into the air supply feeding a burner; the resulting colored flame is photometered by a suitable system of filter or dispersion agent and photocell. The use of this instrument has been found to cut to a small fraction the number of man-hours required to determine sodium or potassium in as diversified samples as inorganic catalysts and fermentation residues.

Another technique which has been made susceptible to quantitative analysis in the past five years is the electrolysis of solutions. Electroanalysis by electrodeposition has long been familiar; the determination of copper by electrodeposition and subsequent weighing is an essential part of almost every course in quantitative analysis. However, only recently was a technique developed for readily measuring the quantity or coulombs of electric current required in the electrolysis as a measure of the quantity of metal deposited. Lingane, who developed the technique, has called it coulometric analysis (*27*). Important to the development and application of coulometric analysis is polarography, of which more later. The development of the technique of electrolysis at controlled cathode potential has given fresh impetus of the old technique of electrodeposition.

However, not all development has been confined to so-called physical methods. Considerable work has been done in the past two decades by Feigl, Dubsky, Spacu, and others (*13*) in improving the specificity of organic reagents. The goal has been the well-known shelf of ninety-odd bottles, each containing a reagent which would give a color or a precipitate with only one element of the periodic system. This goal is being slowly attained as a result of detailed studies of the factors involved in the molecular structure of reagent, pH, solvent nature, and extraction possibilities. The present status of the field of organic reagents has been well summarized in recent books and in a recent comprehensive symposium (*11*).

Other known analytical techniques which have received extensive development in the past few years and promise to have yet wider applicability in the future include the use of isotope tracers for purposes such as the determination of the composition of natural products, and the use of cryoscopic and ebulliometric methods for the determination of the purity of "pure" substances. Recently, isotopic dilution methods have been described for determining the elemental composition of organic compounds (*16*).

UTILIZATION OF HERETOFORE UNUSED PHENOMENA (NEW ANALYTICAL TECHNIQUES)

It is appropriate to consider next a few of the novel techniques introduced into analytical chemistry within the past two and one-half decades—that is, techniques using or based on chemical reactions or physical phenomena or principles which heretofore either were not used in practical analysis or found practically no use in analysis.

Any physical or chemical property can be used as a means of qualitative identification, quantitative measurement, or both, provided that the property is or can be made specific enough for the purpose at hand. Thus, electrodeposition can be used to separate and determine metals if the potentials at which the metals plate out are sufficiently far apart, say 0.2 volt, in the solution being electrolyzed.

A provocative exemplification of an analytical method based on a specific distinguishing property was developed from the long known fact that the only paramagnetic gases at normal conditions are oxygen (O_2), nitric oxide (NO), nitrogen dioxide (NO_2), and chlorine dioxide (ClO_2). However, it was only in 1940 that Linus Pauling and his associates developed an oxygen meter based on this principle, which was not described in the literature until 1946 (*33*). This instrument gives a continuous indication of the partial pressure of oxygen in a gas and has been used for measuring the oxygen in the vapor space of tank cars carrying hydrocarbons such as butadiene, for determining the oxygen content in catalytic converters after regeneration and purging, and for assaying the oxygen content of anesthetic mixtures. The Pauling oxygen meter is relatively simple. A bob carrying a mirror is suspended in an inhomogeneous magnetic field surrounded by the sample gas. The deflection of the bob in the magnetic field is measured as an optical lever and is an indication of the paramagnetic nature of the surrounding gas and consequently of its oxygen content.

Another application of magnetochemistry to analysis is the determination of carbon in steel by measurement of the magnetic permeability of the sample in an instrument devised by Work and Clark (*46*).

Another novel principle is used in the oxygen meter of the Mine Safety Appliances Co. This instrument uses the depolarizing action of oxygen on hydrogen ions as a means of measuring the partial pressure of oxygen in a gas sample.

Polarography, or amperometry, or the use of the dropping mercury electrode, to give its several names, is another relatively new technique. Originated 25 years ago by Heyrovský while studying anomalies in electrocapillary effects, polarography has become, especially during the past decade, a valuable tool in almost every analytical laboratory. Its wide applicability in both inorganic and organic analysis and its relative simplicity of application have made polarographic analysis justifiably popular.

The Geiger-counter x-ray diffraction spectrometer is another application of known but heretofore unused principles to develop further an instrument of great analytical applicability and possibility. The decrease in time from hours to minutes necessary for a measurement should cause and has caused x-ray diffraction to be even more widely investigated from the viewpoint of its analytical possibilities than it now is. It is perhaps the best means at present available for detecting and determining compounds as such. In contrast, the x-ray absorption photometer can be applied to the determination of elemental composition, independent of the state of the element—free or combined—and of the sample—gaseous, liquid, or solid. In this instrument the fluorescence produced on a suitable screen by x-rays after passage through sample and standard is compared by the use of photomultiplier tubes. This essential difference in the use of the two x-ray techniques is inherent in the nature of the phenomena being observed. X-ray diffraction involves essentially the reflection of energy waves from the planes of atoms within the crystal, and the results are accordingly functions of the unit cell or the molecule. X-ray absorption usually involves only the electrons near the nucleus of the atom and hence the result is a function of the element independent of its valence state or physical state. Neutron diffraction is one of many new techniques which may be of analytical service for qualitative elemental analysis.

Outstanding among the newer techniques is the electrometric method recently described by Jensen and Parrack (*23*). This method involves the measurement of the current change produced by a solution placed in the tank circuit of a high-frequency oscillator. The effects which can be measured involve any change in kind and amount of ions, dipoles, or both. The technique, which involves no intimate contact of the sample solution with any apparatus except the sample container, can be used to determine the end point in titrations as in conductometric titrations, to study and measure complex formation, and for many other purposes. The fact that no contact is made with the solution and that so many types of effects can be measured should make this new technique justifiably popular and useful.

Significantly, contemporary analytical chemistry stresses such factors as the use of small samples, the measurement of trace amounts, the nondestructive nature of the analytical process in reference to recovery of the sample, and the specific discriminatory measurement possible. Such factors have been of the most vital importance in the study of, for example, the antibiotics such as penicillin.

Among the newer chemical reactions which are being utilized extensively in analytical work, a prominent place must be given the Karl Fischer and Diels-Alder reactions. The Karl Fischer reagent consisting of a solution of sulfur dioxide, iodine, and pyridine in methanol is being extensively used for the determination of water in all types of material. Bryant, Smith, and Mitchell, in an excellent series of papers which have been summarized and amplified in a recent book (*30*), have extended the use of this reaction to the determination of many types of organic and inorganic compounds which react with or produce water.

The Diels-Alder reaction, involving the condensation of a conjugated diene system in organic compounds with certain olefin systems, has had great applicability in the determination by reaction with maleic anhydride of butadiene and of long-chain fatty acids containing conjugated diene systems. Recently, Bond (*4*) described the use of nitrogen tetroxide in the determination of olefin unsaturation of hydrocarbons, a method which has great promise for this rather difficult determination. The use of relative rates of reaction as an analytical tool as seen in the recent work of Kolthoff and Lee (*24*) seems very promising; such phenomena are the basis of the well-known separation of olefins by absorption in sulfuric acid solutions of varying concentrations.

Other recent developments, which are destined for more extensive use in analytical chemistry, include the measurement of specific dispersion, now widely used in determining the classes of hydrocarbons in petroleum products; electrophoretic methods; ion-exchange and adsorption techniques for separation, purification, and analysis; the use of sound waves in gas analysis; and the application of statistics for evaluating analytical methods and results.

INVESTIGATION OF FUNDAMENTALS OF ANALYTICAL TECHNIQUES AND METHODS

Of great significance to analytical chemistry and analytical chemists have been the fundamental investigations of analytical reactions, procedures, techniques, and apparatus made in recent years. These studies have been the type of study of analytical methods which has been discussed several times within the past few years by the editors of the former Analytical Edition of Industrial and Engineering Chemistry, now more fittingly called Analytical Chemistry. Examples of such investigations are recent papers on the essential experimental factors involved in the measurement of the soft beta-radiation produced by carbon 14, sulfur 35, and other radioactive elements which are extensively used in chemical tracer studies (*26*); on the design of experiments for developing new analytical methods (*44*); on the applicability of microwave spectra to chemical analysis (*10*); on the role of the pressure broadening effect in the infrared spectrophotometry of light gases (*8*); and on the evaluation of accuracy in photometric methods (*1*).

Outstanding examples of programs of investigation of the fundamental background of analytical chemistry are those of Furman, Willard, and their co-workers on the use of cerate solutions as standard oxidizing agents; of Kolthoff and his co-workers on the phenomenon of coprecipitation; and of Mellon and his co-workers on colorimetric methods. Kolthoff used radioactive materials and dyes to investigate the factors involved in the aging of precipitates of analytical interest; the results obtained are the bases of our present concept of entrainment. Mellon, using the Hardy spectrophotometer, has investigated the various factors which influence the color produced in many typical colorimetric reactions.

It is essential that more fundamental and systematic work be done on the nature of analytical techniques and reactions. The importance and necessity for research and development in analytical chemistry are due to the fact that the implementation of analytical chemistry as a technique has far outdistanced the knowledge of its theoretical basis as a science. The early and immediate success of many an analytical technique or method when first used, has tended to obscure the fact that our real knowledge of the applicability and limitations of the method was slight. Sooner or later, this has resulted in difficulty and occasionally in discarding a method which could have been of great service if it had been adequately investigated.

PREPARATION OF PURE COMPOUNDS FOR CALIBRATION AND STANDARDIZATION

One, perhaps not so widely publicized, phenomenon has been the stress laid in recent years on the preparation of pure compounds for calibration and standardization. The analyzed samples for general inorganic analysis and, more recently, for microorganic analysis issued by the National Bureau of Standards are well known for the tremendous value they have been to analytical chemistry. Chemistry—both research and industrial—owes much to G. E. F. Lundell for his devotion in building up this standard sample program.

Because the great majority, if not almost all, of the recent techniques which have entered analytical chemistry, depend upon previous calibration or standardization with the compound being determined, great impetus has been given to the preparation of pure compounds for use as analytical standards. During the war, the National Bureau of Standards and the American Petroleum Institute undertook a huge program involving the preparation of pure compounds for use as analytical standards for the calibration of infrared and ultraviolet spectrophotometers and mass spectrometers. These hydrocarbon samples are now part of the standard sample program of the National Bureau of Standards. In connection with this program, the two organizations have undertaken to issue critical catalogs of physical properties and of ultraviolet and infrared absorption spectrograms for hydrocarbons and for other organic and a few inorganic compounds of interest to the general organic chemical industry as well as to the petroleum industry.

Reference must also be made to the collections of infrared spectrograms issued by the American Cyanamid Company (*2*), the pioneering collections of data on the x-ray diffraction of inorganic compounds issued by the Dow Chemical Company (*19*) and by the American Society for Testing Materials the recent tabulations of azeotropes and of vapor-pressure data published by Dow (*22*, *41*), and the collection of Raman data made available by Fenske and his co-workers (*14*).

DEVELOPMENT OF MORE EFFICIENT SEPARATION TECHNIQUES

Linked, inseparably, to the preparation of pure compounds and to the general process of analytical chemistry are methods of separation. Increasing stress is being placed on improved methods of separation, from the viewpoint of both analysis and the preparation of pure materials. Synthetic organic chemistry is to a great extent dependent upon the ability of an investigator to separate the mixtures resulting from his syntheses. Furthermore, it is necessary, in many cases, to assay the purity or homogeneity of each fraction separated and to identify the pure compounds found. To quote two Nobel Prize winners (*42*): "The biochemist is confronted with separation problems almost daily, and in many cases such separation methods form a key to the solution of his problems"; "No other discovery has exerted such a great influence and widened the field of investigation of the organic chemist as much as Tswett's chromatographic analysis."

Recent years have seen a number of significant additions and modifications to the available separation processes. In many cases, the newer separation techniques serve the same purpose as the older ones such as precipitation. This purpose is, essentially, the preparation of an item that can be measured free of interference. Thus, aromatics can be removed from a mixture of paraffins and naphthenes by adsorption on silica gel, or a C_4 cut suitable for infrared butadiene determination can be secured by fractional distillation.

Connected to the preparation of pure materials and to separation processes are methods of determining the purity and homogeneity of so-called pure compounds or substances. Such methods are absolutely essential for measuring the efficiency of the preparation or separation process used and for determining the actual purity of the standards used in chemical, physical, biological, and medical fields. The use of cryoscopic and ebulliometric methods has been mentioned; other important techniques will be indicated.

Some of the more important of the newer separation techniques can be briefly reviewed.

Fractional distillation or rectification, in large part because of the demands of the petroleum industry, has become a powerful analytical tool, both for the separation of fractions for measurement by other analytical techniques and for analysis per se. The excellent job of instrumentation done by the Podbielniak Company has made available automatic and semiautomatic precision fractional distillation apparatus for the boiling range of −160° to +350° C. In many ways the Podbielniak apparatus represents the first important instrumentation of an analytical technique. A considerable number of packing materials for distillation columns of high efficiency of separation have been developed, such as those of Podbielniak, Fenske, Stedman, and Lecky and Ewell (*35*).

Adsorption means of separation have been extensively developed during the past decade in the well-known field of chromatography of solutions of inorganic and organic substances. Less well known, perhaps, is the work on the analytical separation of gases by fractional adsorption and desorption, such as the Turner-Burrell fractional desorption apparatus for the analysis of mixtures of gases ranging in boiling point from hydrogen to C_5 or higher (*43*).

In the latter apparatus, the sample is adsorbed on a long column of charcoal, along whose length the various compounds arrange themselves roughly in the order of their boiling points, the lowest boiling components being uppermost in the column. Hot mercury is then passed up the column, sweeping out the "column" of gas before it. As each gas comes over from the top of the column, it passes through a thermal conductivity cell whose measurement serves to identify the gas. The volume of the gas is then measured by an interesting device in which the gas, in effect, pushes a small slug of mercury around a standard path, the number of times the slug passes an electromagnetic field in the path being counted. Thus, a record is obtained analogous to that obtained in analytical distillation.

While the low-temperature Podbielniak distillation apparatus plots pressure (quantitative measure) against temperature (qualitative indication), the Turner-Burrell apparatus records volumes in terms of number of circuit-paths traversed (quantitative axis) against thermal conductivity (qualitative axis). Because thermal conductivity does not necessarily increase with the boiling points of the gases, the curve obtained tends to have sharper "breaks," and minor components are more readily detected than in fractional distillation.

Rossini's work on the separation of hydrocarbons using distillation, adsorption, and other techniques is now too well known to need more than mention (*36*, *37*).

In recent years extensive work has been published in Germany by Harteck (*20*) on the fractional desorption of hydrocarbons and in England by Barrer (*3*) on the use of zeolites as molecular sieves in the separation of various types of inorganic and organic molecules.

Lack of space permits only the briefest mention of two valuable separation techniques. Partition chromatography, originally developed by Synge, Martin, and their co-workers (*28*), is the first practical application to analytical chemistry of the principle of continuous countercurrent liquid-liquid extraction. The other technique is countercurrent distribution by discrete stages as developed by Craig and by Trenner (*9*), probably one of the most effective tools at our disposal for separation and testing of homogeneity. Countercurrent distribution was practically the standard method used to evaluate the homogeneity of preparations in connection with the national antimalarial and penicillin programs.

CONCLUSION

In conclusion, a few words should be said on what the author believes to be the important function of the analytical chemist in a large research organization, where the analytical work may be done not only by analytical chemists but also by physical and other classified chemists, physicists, electrical engineers, and others. One particular duty of the analytical chemist is the interpretation of analytical results for which he presumably has been fitted by his training and general chemical experience.

The analytical chemist, as has been often indicated, must act as chemical adviser to the nonchemical groups in the typical industrial research organization. By virtue of his analytical consulting and service work, he usually has greater contact than other chemists with nonchemical groups in aiding the latter to obtain the information they desire in the most economical fashion and in helping them to use that information in furthering their own work. He should help others in his organization plan their experiments and schedule their analyses so as to get the maximum amount of information for the minimum expenditure of time and effort. It is a healthy sign that in many research organizations the analytical chemist is an integral part of the initial research team; he is called in at the beginning in order to aid in planning the work and to start the development of the necessary analytical methods. This is a far different picture from the more usual one where the analytical chemist is not acquainted with the research project until the first batch of samples (naturally marked "Rush") arrives.

The analytical chemist should have a synoptic knowledge of analytical techniques and their applicability, including—most importantly—their limitations, so that, given an analytical problem, he can make a reasonably good decision as to the most fruitful approach to the problem at hand. He must have, if not a working knowledge, at least an awareness of the applicability and limitations of methods, techniques, facts, and instruments of every conceivable type. In solving analytical problems, he must bring to bear on the situation many streams of knowledge; in devising analytical techniques, he must be alert to the possible development of any device or means of measuring chemical reactivity or physical behavior into a means for determining composition.

In approaching an analytical problem, the analytical chemist must have the background and ability to evaluate possible analytical methods from the viewpoints of interference, range of applicability, necessary modifications, and preliminary treatment, as well as the valid interpretation of the experimental measurements obtained. He must know not only how to use the multitudinous analytical techniques at his disposal, but when to use them. In contemporary analytical chemistry it is essential that all approaches and techniques be coordinated on any specific analytical problem in order to obtain the maximum possible information. For example, to determine the nature of the totally unknown ash obtained on the incineration of an organic compound, the logical procedure might be to use emission spectroscopy to obtain an identification of the elements in the ash and then to determine the individual elements by colorimetric, polarographic, gravimetric, or other methods depending on the amounts of the elements present; the information obtained could then be rounded out by the use of x-ray diffraction and microscopy to determine the compounds actually present in the ash.

The training of the chemist for contemporary analytical research and chemical analysis must be broad and thorough. Within the inherent limitations of time and energy, the prospective analytical chemist should try to acquaint himself with as broad an area of chemistry and of pertinent aspects of physics, electronics, and optics as feasible. One of the most serviceable fundamental bases for research in analytical chemistry is a thorough indoctrination in the methods, techniques, and approaches of so-called classical gravimetric and titrimetric analysis. A comprehensive training in these fields gives one the basic attitude necessary for the most expeditious solution of analytical problems. In approaching an analytical problem, for example, a background in mineral analysis teaches one how to judge possible analytical methods from the viewpoints of interferences, necessary preliminary treatment, and valid interpretation of the experimental measurements obtained. The man with good basic academic training, given the necessary intellectual qualifications, can readily learn how to use—or at least, and perhaps more importantly, when to use—the multitudinous analytical techniques now at our disposal. The analytical chemist should be taught principles, not methods; he should learn "instrumentation," not "instrumental analysis."

In closing, it might be pertinent to quote Beverly Clarke again (*6*): "The analyst of today uses constantly, to a greater extent perhaps than workers in any other branch of chemistry, the fact and theory of all the subdivisions of chemical science."

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No attempt has been made to include all pertinent references; instead, reference has been made largely to review or summary papers. In some cases, older papers of historical significance have been cited in preference to more recent papers. The review issues of ANALYTICAL CHEMISTRY in January 1949 and January 1950 are good general sources of reference to the phenomena cited. Strong's survey (*40*) of analytical papers published in 1946 is a pertinent statistical classification of current trends. The author will be pleased to supply specific references for any of the items discussed.

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Infrared Analysis of Phenol, Cresols, Xylenols, and Ethylphenols

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QUANTITATIVE infrared analyses of phenol and C_7 and C_8 alkyl phenols in coal-hydrogenation oil products have been carried out. These determinations are important for by-product evaluation in production of synthetic liquid fuels. The spectra of various C_7 and C_8 alkyl phenols in the sodium chloride region have been published by Barnes, Gore, Liddel, and Williams (*1*) from 5 to 10 microns, by Kletz and Price (*3*) in liquid and solid states, and by Whiffen and Thompson (*4*) as carbon disulfide solutions. The latter authors reported analyses of cresols but only discussed the possibility of analysis of xylenols and ethylphenols. The present paper describes the analysis of phenolic compounds up to and including the ethyl phenols and four of the six xylenols.

CALIBRATION COMPOUNDS

Sources, purifications, and purities of the various phenols used as calibration compounds are as follows:

Phenol. Baker's C.P., distilled and recrystallized to melting point of 38.8–39.5° C.

o-Cresol. Koppers, purified to melting point of 30.6° C. Estimated purity by freezing point measurements, 99.5%.

Qualitative and quantitative analyses by means of infrared spectrometry have been carried out on carbon disulfide solutions of phenol and C_7 and C_8 alkyl phenol mixtures separated from coal-hydrogenation oil. The base-line method of calculation permitted accurate quantitative results to be obtained from the original curves used for qualitative interpretations. Measurements on six bands used in analysis disclosed that deviations from Beer's law linearity were practically zero. Data are presented showing the results obtained on synthetic blends as well as on the actual samples.

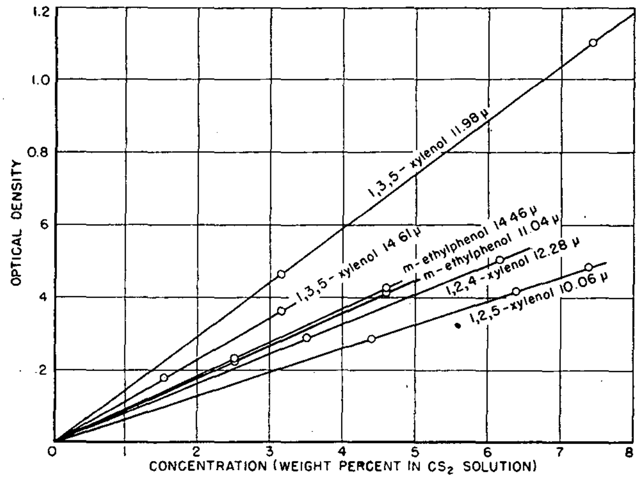

Figure 1. Obedience to Beer's Law

m-Cresol. Koppers, purified to melting point of 12.0° C. Estimated purity by freezing point measurements, 99.0%.

p-Cresol. Koppers, purified to melting point of 33.2° C. Estimated purity by freezing point measurements, 97.9% (impurity found spectrometrically, 1.9% *m*-cresol).

o-Ethylphenol. Koppers, prepared by ethylation of phenol. Distillation produced a heart cut with boiling point of 204.5° C. (760 mm.). Purified to melting point of −24.3° C.

m-Ethylphenol. Reilly. Distillation produced a heart cut with boiling point of 218.5° C. (760 mm.). Purified to melting point of −3.4° C.

p-Ethylphenol. Koppers, prepared by ethylation of phenol. Distillation produced a heart cut with boiling point of 218° C. (760 mm.). Purified to melting point of 45.1° C.

1,2,6-Xylenol. Edcan Laboratories. Distillation produced a heart cut with boiling point of 201° C. (760 mm.) and melting point of 45° C.

1,2,4-, 1,2,5-, 1,3,4-, and 1,3,5-Xylenols. Eastman Kodak White Label, redistilled through a 27-plate column to obtain heart cuts. The infrared spectra are nearly identical to spectra of the corresponding compounds by Whiffen and Thompson (*4*).

EXPERIMENTAL

The spectrometer used is a conventional Perkin-Elmer Model 12B with direct current thermocouple, General Motors breaker-type amplifier, and Brown Electronik recorder. Resolution throughout most of the rock salt spectrum is believed equal to that of similar models; but, with the present thermocouple and optical alignment, resolution in the long wave-length region is unexpectedly high. The 15.0-micron carbon dioxide fine structure, with band separations of about 1.6 cm.$^{-1}$, can be sharply resolved at normal scanning speed (slit width, 0.200 mm.).

Deviations from Beer's law linearity are decreased by use of small slit widths made possible by this resolution. Measurements on six bands for four of the main constituents disclosed that deviations from linearity were all practically zero (Figure 1). Scattered light usually causes nonlinearity, especially at long wave lengths, but did not in the present work because of the resolution and the optical density range. Less resolving power will permit sufficiently accurate analyses if concentrations are kept low enough for approximate linearity, or if appropriate corrections are made for nonlinearity.

Calibration spectra for the pure phenols were measured in a 0.1-mm. cell in carbon disulfide solutions ranging from 7.5 to 1.5 weight % phenol. Synthetic blend and sample spectra were obtained mostly at concentrations of 15 weight %.

The noise level maintained was less than ±0.1% of full scale deflection. Large deflections were utilized throughout, except in the 14.5-micron region where deflections with carbon disulfide in the cell were 25% of full scale (slit width, 0.340 mm.).

PROCEDURE AND RESULTS

The phenols were extracted (*5*) from the *n*-hexane-soluble portion of coal-hydrogenation oil prepared by hydrogenating, at 3500 pounds per square inch (246 kg. per sq. cm.) and 440° C., a slurry of 40% Pittsburgh-bed coal, 59% recycle oil, and 1% molybdenum trioxide catalyst. The extract was fractionated at low pressure into 135 cuts, of which the first 60-odd cuts were found to contain C_8 phenols and lower. Comparison of the spectra of these lower cuts with the spectra of pure compounds permitted qualitative determinations of phenols present.

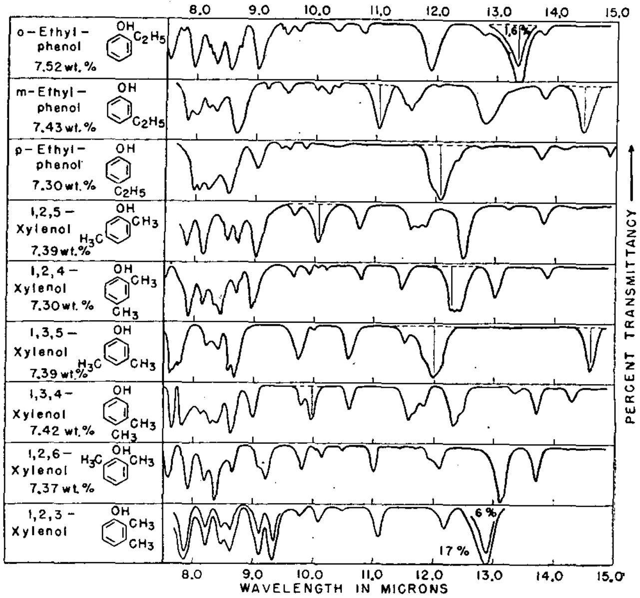

Figure 2. Infrared Spectra of C_8 Alkyl Phenols in Carbon Disulfide Solutions

Absorption bands and base lines used in analysis are indicated. Spectrum of 1,2,3-xylenol from (*4*)

The method of quantitative analysis, the base-line method of Wright (*6*) and Heigl, Bell, and White (*2*), was chosen because: (1) spectral bands of phenols are mainly symmetrical and widely spaced to permit easy choice of base line, (2) spectra of the pure solvent are not required because accurate transmittancy values are not needed, and (3) qualitative and subsequent quantitative results are obtained from the original spectrum of the unknown

Table I. Infrared Analyses of Synthetic Blends of C_8 Alkyl Phenols in Carbon Disulfide Solutions

	Blend											
	I		II		III		IV		V		VI	
Components	Syn.	Anal.	Syn.	Anal.	Syn.	Anal.	Syn.	Anal.	Syn.	Anal.	Syn.	Anal.
1,2,4-Xylenol	50.4	50.4	40.5	41.1	10.5	11.0	25.0	26.2	...	...	...	...
1,2,5-Xylenol	...	...	41.0	41.5	9.9	9.2	25.0	24.3	...	...	...	...
m-Ethylphenol	41.8	40.5	8.9	8.4	40.1	41.8	...	...	42.0	42.9	...	...
o-Ethylphenol	7.9	9.1	9.8	9.0	39.4	38.0	...	...	...	...	...	...
p-Ethylphenol	...	...	...	...	...	...	...	...	...	...	49.9	49.5
1,3,5-Xylenol	...	...	...	...	...	...	25.0	23.4	38.6	38.3	50.1	50.5
1,3,4-Xylenol	...	...	...	...	...	...	25.0	26.1	19.4	18.8	...	...

Table II. Absorption Band–Concentration–Slit Width Data Used for Infrared Analyses of Phenolic Mixtures

	Fractions					
	1–24	25–43	44–51	52–55	56–62	63–71
Components	Absorption Band (Microns)—Concentration (Weight Per Cent)					
Phenol	9.36–7.43	9.36 –7.43	...	...	...	...
o-Cresol	9.03–2.42	13.31[a]–2.42	...	...	...	...
p-Cresol	...	12.18[a]–2.49	...	...	...	...
m-Cresol	...	12.90 –2.35	14.55–2.35	14.55–2.35	...	...
1,2,4-Xylenol	...	...	12.28–7.30	12.28–7.30	...	...
1,2,5-Xylenol	...	...	10.06–7.39	10.06–7.39	...	...
o-Ethylphenol	...	...	13.35–1.60	13.35–1.60	13.35–1.60	...
p-Ethylphenol	...	...	12.08–3.65	12.08–3.65	12.08–3.65	...
m-Ethylphenol	...	...	14.46–3.66	11.04–7.43	11.04–7.43	11.04–7.43
1,3.5-Xylenol	...	...	...	11.98–3.70	11.98–3.70	11.98–3.70
1,3,4-Xylenol	...	...	...	...	9.98–7.42	9.98–7.42

Slit widths 8.0 to 9.2μ = 0.105 mm.
9.2 to 11.2μ = 0.140 mm.
11.2 to 12.8μ = 0.185 mm.
12.8 to 13.9μ = 0.240 mm.
13.9 to 15.0μ = 0.340 mm.

[a] Usable if phenol is low.

without need of further measurements. Spectra of the six xylenols and the three ethylphenols from 7.5 to 15.0 microns are shown in Figure 2. The three ethylphenol spectra are very similar to the corresponding cresols (*4*), as expected. Also indicated in this figure are the absorption bands and base lines principally used for analysis. In the actual analysis the spectra were not converted to the transmittancy plots of Figure 2, but, rather, base lines were drawn on the recorded "sawtooth" curves.

The first step in the quantitative analyses was the preparation of synthetic blends to attempt simulation of the phenolic mixtures as based on rough quantitative estimates from the original spectra. The analyses of known blends of C_8 alkyl phenols are shown in Table I. Blends of phenol and *o*-, *m*-, and *p*-cresols, not included in the table, also were analyzed and with somewhat better accuracy than that obtained for the C_8 phenols. In all analyses base lines were drawn as nearly as possible in a manner corresponding to the way they were drawn from the pure compounds—that is, between regions of no specific absorption. This was relatively easy for the phenols because of the wide separation of bands. In some cases the proper position of the base line was not certain without meticulous examination of all the calibration spectra involved. A short-cut answer was obtained by back-calculation of the optical density for the mixture from the concentrations as synthesized, followed by calculation of correct base-line position.

Table III. Infrared Analyses of Phenolic Fractions from Coal-Hydrogenation Oil in Carbon Disulfide Solutions

Components	Fractions, Weight %								
	1–3	4–7	8–20	21	22–24	25–30	31–34	35–40	41–43
Phenol	100.0	96.8	61.9	26.7	8.8	0.9	...	...	...
o-Cresol	...	3.2	38.1	73.3	91.2	34.8	12.3	4.8	2.3
p-Cresol	...	..	..	..	...	23.3	27.2	31.4	34.7
m-Cresol	...	..	..	..	...	41.0	60.5	63.8	63.0

	Fractions, Weight %								
	44–47[a]	48–51	52–55	56–61	62[b]	63–65[c]	66[c]	67[c]	68–71
m-Cresol	12	6	5	...	...	...	..	..	...
1,2,4-Xylenol	56	35	10	...	...	...	..	..	...
1,2,5-Xylenol	30	24	9	...	...	...	..	..	...
o-Ethylphenol	2	7	3	2	1	...	..	..	...
p-Ethyl phenol	(−1.2)	8	16	14	3	...	..	..	...
m-Ethyl phenol	(−0.8)	20	54	65	33	(36)	(12)	(1)	...
1,3,5-Xylenol	...	...	3	19	63	(47)	(40)	(30)	(10)
1,3,4-Xylenol	...	...	...	...	(−0.4)	...	..	..	(15)

[a] Mother liquor from extraction of 1,2,4-xylenol.
[b] Mother liquor from extraction of 1,3,5-xylenol.
[c] Complete analysis not possible because of interference of unknown C_9 alkyl phenols. Percentages of *m*-ethylphenol, 1,3,5-, and 1,3,4-xylenol are approximate.

Calibration wave lengths, concentrations, and slit widths used in analysis of the unknown fractions are given in Table II. Results for the various fractions or combined fractions are presented in Table III. Negative values indicate that the compounds were absent or present in concentrations less than the experimental error of the method. Analyses of fractions 63 to 71 are incomplete because of interference of unknown C_9 phenols, but the approximate concentrations of 1,3,4- and 1,3,5-xylenol and of *m*-ethylphenol are believed valid. 1,2,6- and 1,2,3-xylenols have not been detected; they are known to be present only in traces in coal-tar phenols.

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Determination of Isotope Ratios of Known Deuterium-Hydrogen Samples Using a Mass Spectrometer

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Gas samples of various known deuterium enrichments were prepared by reducing deuterium oxide–water mixtures over zinc. The mass 3–mass 2 ratio for each of these standards was obtained on the Consolidated-Nier Model 21-201 mass spectrometer. The measured ratio was then plotted against known enrichment. The resulting calibration curve can be used to determine absolute enrichments of unknowns from their measured ratios. This method of calibration permits setting all instrumental factors for maximum stability and precision, regardless of whether or not this setting yields the absolute ratio. The curve obtained can be used for one instrument over a considerable period of time under various instrumental conditions.

IN ATTEMPTING the determination of deuterium-hydrogen ratios by the mass spectrometer several difficulties were encountered. Various investigators (*6*) also had reported difficulties in the attempt to obtain deuterium-hydrogen ratios of maximum precision and accuracy.

This paper describes a method of preparing samples of known deuterium enrichment, and a satisfactory procedure for using them to determine the isotope ratio of unknown samples by mass spectrometric methods.

EXPERIMENTAL

Eight standard solutions were prepared by diluting 99.8% deuterium oxide with doubly distilled water to give the following (mole per cent of deuterium oxide in excess of that ordinarily found in distilled water): 0.01, 0.03, 0.04, 0.3, 0.5, 1.0, 1.1, and 1.2%.

These samples, and a sample of doubly distilled water, were then reduced over zinc at 400° C. according to the method of Rittenberg (*11*). The best reduction was obtained using commercial 10-mesh zinc previously treated with 25% hydrochloric acid, thoroughly washed with water, alcohol, and ether, and dried in an oven at about 80° C. Using a quartz tube 50 mm. long with an inside diameter of 11 mm., containing about 55 grams of zinc, approximately eight 20-mg. (0.02-ml.) samples could be reduced before the zinc needed replacement. The mixture of hydrogen-deuterium formed was collected in a sample container over mercury in a previously evacuated system (*1*).

The flask was then attached to the mass spectrometer, and mass 3–mass 2 ratios (HD/H_2) were read for various magnitudes (in volts) of the mass 2 peak. The instrument used was the Consolidated-Nier Model 21-201 mass spectrometer (*8*, *13*). The double exit slit system of this instrument, designed to handle isotopes of carbon, nitrogen, and oxygen, cannot be used for hydrogen isotopes because the mass 2 (HH^+) and mass 3 (HD^+) ion beams are too far apart to be simultaneously collected. This necessitates focusing first the mass 2 ion and then the mass 3 ion on one collector (collector 1 for the data that follow) and measuring the ratio of the voltages developed (*5*). The magnetic scan method was used to avoid the voltage effect (*7*).

The necessity of measuring the two peaks at different times reduces the precision of hydrogen measurements in comparison with the heavier materials such as carbon dioxide and nitrogen. Results obtained in this investigation were reproducible to about ±3% of ratio over a period of 2 weeks, and to ±1% of ratio or better over short periods of several hours.

Both the value of the HD/H_2 ratio and its reproducibility are affected by instrumental factors, particularly the setting of the source magnet (*2*). Therefore all instrumental variables were set so as to obtain optimum precision rather than absolute accuracy.

It was found that after admission of samples enriched with deuterium in the range of 0.01 to about 1%, it was necessary to evacuate the tube by pumping for 25 minutes in order to eliminate the "memory effect." [This phenomenon has been discussed elsewhere (*9*, *13*).] This time could be somewhat shortened by flushing with normal hydrogen several times during the pump-out period. After a sample of 25 atom % enrichment had been passed through the instrument for 20 minutes, 4 hours of alternate pumping and flushing with normal hydrogen were required before the ratio of standard hydrogen returned to normal. Flushing with helium did not accelerate elimination of the memory effect.

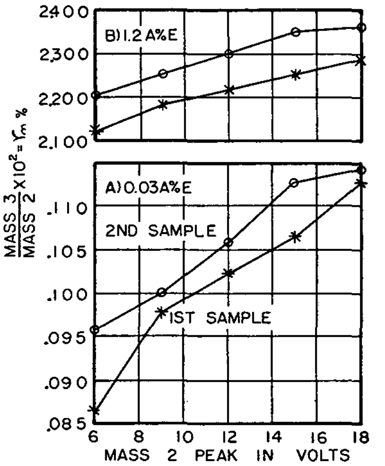

Figure 1. Fractionation during Reduction of Water to Hydrogen-Deuterium Gas

All samples were tested for the presence of air and water by checking the 32 and 18 peaks, respectively (*5*). The presence of air increases the mass 3–mass 2 ratio; the plot of ratio against pressure has a greater slope when air is in the sample. The presence of water possibly leads to exchange reactions with deuterium present. Therefore, in this work, samples showing 32 or 18 peaks greater than 0.002 volt above the instrumental background voltage were discarded.

DISCUSSION AND RESULTS

The data obtained for tank hydrogen, hydrogen from unenriched doubly distilled water, and the eight synthetic samples are shown in Table I. The 0.03 and 1.2 atom % enrichment samples show the effect of fractionation during the reduction process (Figure 1). In these cases sufficient sample (0.04 ml.) was reduced to fill two sample containers completely. In each case the second sample collected has a higher mass 3–mass 2 ratio than the first; hence, to ensure good results, the water sample used must be completely reduced. With all the other samples this precaution was taken. In the 0.03 and 1.2 atom % enrichment samples, the average atom per cent was used. Distilled water was taken as the base in computing enrichments, so that the deuterium oxide added was the actual excess.

The ratios obtained on the mass spectrometer should be multiplied by the factor $(2/3)^{1/2}$ because of the difference in pumping speed of materials of different masses from the source. The leak is so designed that gases of all masses enter at the same rate. However, hydrogen pumps out more rapidly than HD, so that all ratios are high by the factor $(3/2)^{1/2}$. In the calibration method described below, it is not necessary to apply this correction.

The formula used in computing atom per cent from measured mass 3–mass 2 ratios, r, was (*10*):

$$A_m\%/100 = A_m = \frac{r}{2 + r}$$

where $r = \dfrac{r\%}{100}$

The measured ratio rises with pressure, presumably because of the presence of H_3^+ ions (*3*, *9*, *13*). The computed enrichment also increases with pressure; therefore, subtracting the standard does not completely compensate for the effect of H_3^+. To nullify this interference of H_3^+ with the mass 3 ion HD^+, the mass 3–mass 2 ratio is plotted against pressure. In this case the ratio is plotted against the magnitude of the mass 2 peak in volts, inasmuch as the voltage, or peak magnitude, is directly proportional to pressure. The plot, for small enrichments, is linear, and can be extrapolated to the zero-pressure ordinate. The ratio at this point, corrected by the pumping factor of $(2/3)^{1/2}$, should be very nearly the absolute ratio, barring instrumental bias in collection of the ions of the two masses. Table II shows a comparison between known enrichments and those calculated by extrapolating in this manner from the data of Table I. The deviations are considerably greater than can be explained by the expected variations in either the preparation of the samples or the measurement of ratios. They are only partially explained by the fact that the intercepts are somewhat in doubt, particularly in larger enrichments, because of deviations from linearity—curves for 1.0, 1.1, and 1.2 atom % enrichment being concave downward.

Table I. Mass 3–Mass 2 Ratios for Synthetic Samples at Various Source Pressures

Atom % Enrichment Known from Synthesis	Source Pressure (Peak 2, Volts)	r_m % Measured, on Mass Spectrometer	Atom % Computed from r_m %	Computed Atom % Enrichment
... (tank H_2)	6	0.0259	0.0129	....
	9	0.0280	0.0140	....
	12	0.0343	0.0171	....
	15	0.0383	0.0191	....
	18	0.0418	0.0209	....
0.00 (hydrogen from doubly distilled water, normal)	6	0.0393	0.0196	....
	9	0.0440	0.0220	....
	12	0.0469	0.0234	....
	15	0.0527	0.0263	....
	18	0.0558	0.0279	....
0.01	6	0.0533	0.0266	0.0070
	9	0.0583	0.0291	0.0071
	12	0.0663	0.0331	0.0097
	15	0.0683	0.0341	0.0078
	18	0.0733	0.0366	0.0087
0.03	6	0.0861 0.0955	0.0454 (mean)	0.0258
	9	0.0980 0.1000	0.0495	0.0270
	12	0.1024 0.1054	0.0519	0.0285
	15	0.1063 0.1123	0.0546	0.0283
	18	0.1125 0.1140	0.0566	0.0287
0.04	6	0.1108	0.0554	0.0358
	9	0.1185	0.0592	0.0372
	12	0.1215	0.0607	0.0373
	15	0.1295	0.0647	0.0384
	18	0.1331	0.0665	0.0386
0.30	6	0.540	0.270	0.250
	9	0.580	0.290	0.263
	12	0.595	0.297	0.274
	15	0.600	0.300	0.274
	18	0.620	0.310	0.282
0.50	6	0.856	0.428	0.408
	9	0.890	0.445	0.423
	12	0.934	0.467	0.444
	15	0.945	0.472	0.446
	18	0.961	0.480	0.452
1.0	6	1.745	0.865	0.846
	9	1.811	0.897	0.875
	12	1.860	0.921	0.898
	15	1.872	0.927	0.901
	18	1.901	0.942	0.914
1.1	6	1.896	0.939	0.919
	9	1.962	0.971	0.949
	12	1.999	0.990	0.966
	15	2.019	0.999	0.973
	18	2.023	1.002	0.974
1.2	6	2.124 2.204	1.070	1.051
	9	2.181 2.251	1.096	1.074
	12	2.217 2.298	1.116	1.093
	15	2.252 2.344	1.136	1.109
	18	2.285 2.360	1.148	1.120

Table II. Ratio Extrapolated to Zero Pressure to Eliminate H_3^+

Sample	r_m %	r_m %$(2/3)^{1/2}$	*A*%	*A*%*E*
Tank H_2	0.0158	0.0129	0.00644	....
Distilled H_2O	0.0311	0.0254	0.0127	....
0.01 A%E	0.0432	0.0353	0.0176	0.0049
0.03 A%E	0.0841	0.0687	0.0343	0.0216
0.04 A%E	0.1005	0.0821	0.0410	0.0283
0.3 A%E	0.520	0.425	0.212	0.199
0.5 A%E	0.804	0.656	0.327	0.314

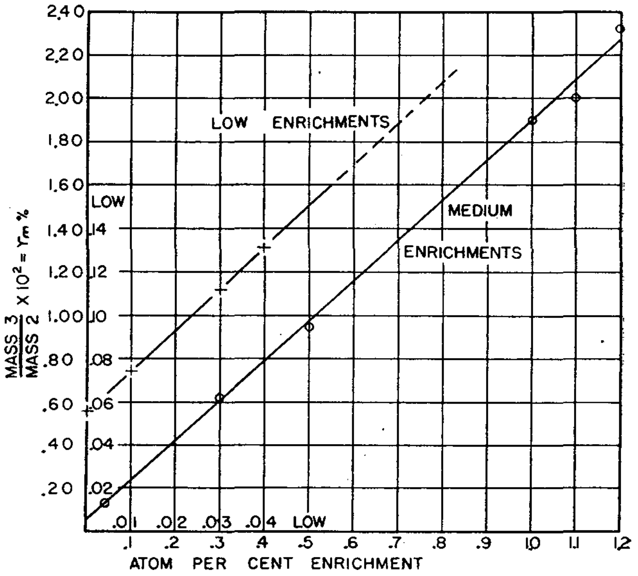

Figure 2. r_m vs. Atom % Enrichment at 18 Volts of Mass 2

It was concluded therefore, that instrumental factors, such as source magnet placement, alter relative collection efficiencies at masses 2 and 3; hence the synthetic samples were used to construct a calibration curve for the instrument (Figure 2). Ratios read at an arbitrary sample pressure in the mass spectrometer are plotted as the ordinate, the enrichment or excess over doubly distilled water as the abscissa. The standard pressure chosen was 18 volts of the mass 2 peak, although any value above 12 volts would be equally useful. Below 12 volts, however, the precision begins to drop because of the very small size of the mass 3 peak.

The data are plotted to two scales, to permit reading to sufficient accuracy in the region below 0.05 atom % enrichment. The curve is linear over the range from 0 to 0.04 atom % enrichment, and linear within limits of experimental error over the entire range of 1.2 atom % enrichment.

When an unknown sample is run, the standard sample closest to it in ratio is also run. The measured ratio from this standard is plotted against its known enrichment on the calibration sheet.

If this point falls on the established curve, the atom per cent enrichment corresponding to the ratio obtained for the unknown sample is read directly from the curve. If the ratio of the standard falls slightly to one side of the curve, a line is drawn through the new point parallel to the calibration curve. The enrichment corresponding to the ratio obtained for the unknown is then read from this corrected segment of curve. Thus, the calibration curve can be used for a considerable period of time, over somewhat changing operating conditions.

Using the method of calibration permits adjusting the instrument itself for maximum stability, regardless of its effect on the measured value of the ratio. Deviation of measured from absolute ratios will then be determined by the accuracy of synthesis of the standards and by the reproducibility of the instrumental measurements. By using adequate precautions in preparing standards, therefore, this method of calibration results in accuracy approaching instrumental precision.

Of particular interest in future comparisons of data obtained by various laboratories is the large difference between the HD/H_2 ratio of unenriched tank hydrogen and that obtained from reduction of unenriched doubly distilled water. Tank hydrogen contains only one half to two thirds as much deuterium as does the hydrogen from water. Furthermore, variations in deuterium content of hydrogen and water samples from various sources have been reported (*4*, *12*). It is true that any arbitrary standard can be chosen for one laboratory, but obviously the base isotope concentration must be known and specified if data from different laboratories are to be compared.

SUMMARY AND CONCLUSIONS

A satisfactory procedure for determination of deuterium-hydrogen mixtures has been developed using the Consolidated-Nier 21-201 mass spectrometer. The minimum detectable difference from normal distilled water when frequent standards are run is 0.0004 atom %, or less under favorable conditions. Over the entire range, reproducibility is ±1% of the ratio for periods of several hours and ±3% of the ratio for longer periods.

It was found that instrumental factors, including the setting of the source magnet, affect materially the value of the ratio of HD/H_2 obtained. Hence, the instrument was calibrated with synthetic samples of known deuterium enrichments, hydrogen obtained from doubly distilled water being used as zero enrichment. In preparing the calibrating samples, it was found necessary to collect hydrogen from the entire water sample placed in the reduction apparatus, as a different ratio was obtained from the first and last half of the reduction. The calibration curve showing known enrichment plotted against measured HD/HH ratio can be used for a range of operating conditions and hence for an extended period of time and has the advantage of yielding results in nearly absolute enrichment.

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Determination of Certain Ortho-Substituted Phenols

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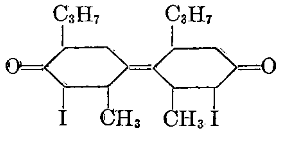
A colorimetric method for the determination of *o*-phenylphenol or *o*-*tert*-butylphenol in the presence of their para isomers is based on the formation of their aristols and subsequent extraction with toluene. The optimum amount of *o*-phenylphenol is about 2.5 mg. and of *o*-*tert*-butylphenol is about 0.5 mg. An accuracy of about 1% is practical.

SYNTHETIC coating resins are prepared in large volumes from *p*-*tert*-butylphenol and from *p*-phenylphenol. One of the most objectionable impurities to be found in these *p*-phenols is the ortho isomer. The *o*-phenol tends to cause a yellow color in the final resin; poor drying characteristics and other undesirable features are also present. The present analytical methods were developed as controls for certain experimental processes, but it is believed, that they may find application in the routine assay of commercial phenols.

In the presence of alkali and iodine, an *o*-alkyl or aryl phenol undergoes iodination and subsequent polymerization. Bordeianu (*1*, *2*) showed that polymerization occurs through the positions para to the hydroxyl groups. He showed that the action of oxidizing agents on 2,4-diiodothymol and 2-iodo-4-bromothymol yielded a red polymer through the same diphenoquinone iodide.



The results of Poplawski (*4*) are in agreement with these. Wollett (*5*) found polymers with molecular weights up to 4400. This necessity for a free para position indicates that a di-*o*-phenol would interfere with the present procedure, but that an *o*,*p*-dialkylphenol would not

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interfere. Emery and Fuller (*3*) described a gravimetric method based on the same reaction, but it distinguishes only qualitatively between *o*- and *p*-phenols.

REAGENTS

Approximately 1.0 *N* hydrochloric acid. Approximately 0.1 *N* iodine solution. Approximately 0.1 *N* sodium thiosulfate solution. Starch indicator. Toluene, reagent grade. Buffer, 0.5 molar aqueous sodium carbonate solution.

PROCEDURE

A weighed sample of the phenol is dissolved in a slight excess of dilute alkali. To an aliquot that contains no more than 10.0 mg. of total phenols and no more than 2.5 mg. of *o*-phenylphenol (or 0.5 mg. of *o-tert*-butylphenol) are added 5.0 ml. of buffer and 10.0 ml. of 0.1 *N* iodine. After 1 minute 10.0 ml. of 1 *N* hydrochloric acid are added. The excess iodine is destroyed with 0.1 *N* thiosulfate and starch, 50 ml. of toluene are added, and the flask is stoppered and vigorously shaken for 30 seconds. The two layers separate readily and a portion of the toluene is decanted through a coarse filter paper into a colorimeter tube. The color is read at 490 millimicrons (at 450 millimicrons with *o-tert*-butylphenol).

EXPERIMENTAL RESULTS

The results reported in Tables I to X are for *o*-phenylphenol; very similar results were obtained for *o-tert*-butylphenol and have been omitted. In each of the experiments a sample containing 2.5 mg. of *o*-phenylphenol was used and the above procedure was followed except for the concerned variable.

Table I. Effect of pH of Buffer

pH	Optical Density	pH	Optical Density
8.4	207	12.0	219
9.7	211	12.5	219
10.5	214		

Table II. Effect of Volume of Buffer

Buffer, Ml.	Optical Density	Buffer, Ml.	Optical Density
1	192	3	219
2	219	5	219

Table III. Effect of Volume of Iodine

Iodine, Ml.	Water, Ml.	Optical Density
1.0	4.0	139
2.0	3.0	219
3.0	2.0	220
5.0	0.0	219

Table IV. Effect of Total Volume during Iodination

(After iodination volumes were adjusted to same figure)

Volume during Iodination, Ml.	Optical Density	Volume during Iodination, Ml.	Optical Density
11	219	25	206
20	216	30	196

Table V. Effect of Iodination Time

Time, Min.	Optical Density
0.25	202
0.50	218
1.00	219
5.00	218

Table VI. Effect of Excess Hydrochloric Acid

Excess 1.0 *N* Acid, Ml.	Optical Density
0.0	219
5.0	220
10.0	218

Table VII. Effect of Excess Thiosulfate

Excess 0.1 *N* Thiosulfate, Ml.	Optical Density	
	After 0.5 minute	After 10.0 minutes
0.0	219	219
5.0	218	219

Table VIII. Effect of Volume at Extraction

(Extra water added just before toluene extraction)

Volume at Extraction, Ml.	Optical Density
26.0	219
51.0	220

Table IX. Effect of Volume of Toluene

Toluene, Ml.	Optical Density	Optical Density × Ml. × 10^{-3}
20	472	9.44
30	312	9.36
40	233	9.32
50	188	9.40
60	159.5	9.30

Table X. Standardization Curve

o-Phenylphenol, Mg.	Optical Density	*o*-Phenylphenol, Mg.	Optical Density
0.000	0.0	1.500	126.0
0.500	39.7	2.000	168.5
1.000	79.2	2.500	205.8

DISCUSSION

All the variables in the procedure have been, in so far as possible, adjusted so that minor changes in technique will not affect the results.

Several attempts were made to analyze the color bodies. All results indicated that the product was of varying quinone content. This is in agreement with previous publications as well as the conclusions drawn from experimental data. The reaction is definitely not a stoichiometric one and for this reason details should be standardized as much as possible.

An examination of the experimental data accumulated in the development of this method allows the following conclusions to be drawn: It is permissible for the pH of the buffer to vary in the range of 12.0 to 12.5. Low results will be obtained on either side of this range. The procedure allows a twofold excess of buffer over the minimum necessary.

The volume during iodination must be the same during each determination, for the amount of color body formed decreases as the volume increases. The concentration of iodine specified is twice the minimum amount. The iodination time is not a variable within the range of 0.5 to 5.0 minutes.

Excess hydrochloric acid and sodium thiosulfate have little effect on the color. This is probably due to the extreme insolubility of these aristols (thymol iodides).

This method is not ideal by any means; it requires a skilled technician and close attention to detail. When used as a qualitative test, such care is, of course, not necessary.

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Identification of Organic Dyestuffs by X-Ray Powder Diffraction

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The x-ray powder pattern is used for the identification of organic dyestuffs by comparison with standard samples. A discussion is given of the difficulties arising from polymorphism and the sensitivity and limitation of the method. Application of this technique to various dyestuffs and to difficult problems of dyestuff chemistry is described. The x-ray analysis is compared with methods such as chemical identification, dye tests, and heated specimen microscopy.

THOUGH almost all commercial dyestuffs are crystalline in nature, the crystals are often not visible, even through a microscope. It is known that crystalline materials in powder form give characteristic x-ray diffraction patterns made up of lines in certain positions and of varying intensities. From these lines, the spacing values for different planes of the crystal lattice can be calculated by means of the wave lengths of the radiation. Thus the powder pattern or the spacing values of the crystal lattice are a definite characteristic of any dyestuff.

In this country, J. D. Hanawalt, as early as 1936, suggested the general use of powder patterns for the identification of crystalline materials. He and his co-workers (*12*, *13*) published the first set of diffraction data for 1000 common chemical substances. They have also presented much valuable information on the procedure to be followed for the classification of the diffraction patterns and the possibilities of the x-ray technique as it is applied mainly for inorganic materials. The wider use of the x-ray diffraction method depends upon the availability of a large collection of such data. When these are known, powder patterns may be employed as an effective method for "fingerprinting" crystalline substances, because each gives its own characteristic diagram. The work involved in collecting x-ray data in a standard form is considerable. Up to the present time a joint American and British committee has published x-ray data on about 2500 substances in the form of a card index, but very few organic compounds are among them. This collection is continually being extended (*1*).

Several organic dyestuffs have been investigated by the x-ray method for scientific purposes, but nothing has been published on their identification by this means, although some industrial laboratories make use of the technique. This paper describes the identification of dyes by the x-ray pattern as it has been practiced in Germany since 1933. Until this time no information on it has been available outside the I. G. Farbenindustrie organization where it was used, except for brief summaries of its development (*4*, *8*). Because these methods differed in some respects from those outlined by Hanawalt, the most useful features of both procedures in terms of current demands are indicated.

X-RAY METHODS USED FOR ORGANIC DYESTUFFS

Technique. Most of the powder patterns were made with filtered Cu K α radiation, while a few organic dyestuffs required the use of Fe K α radiation. In place of the normally employed cylindrical camera, special flat cameras were used with which ten to twelve different patterns could be made on the same 9 × 12 cm. (3.5 × 4.7 inch) film. However, only the equatorial part of the power diagram appeared. These cameras were constructed to speed the work and to facilitate comparison of the samples with the standards, as no classification system such as that suggested by Hanawalt was used. Copper radiation and flat cameras are more suitable than molybdenum radiation and cylindrical cameras for organic dyes (*17*). Time of exposure varied from 10 to 20 minutes. Short exposure is generally preferable to obtain quick results and to increase the efficiency of normal equipment. It is often necessary to rotate the samples during exposure to avoid misinterpretation as a result of preferred orientation.

Specimens. Commercial dyes in the form of a powder can be used as specimens for the x-ray examination in the state in which they are received. Pastes should be dried at room temperature in a desiccator after removal of water-soluble ingredients, and then powdered. Special purification is not necessary, as the diffraction patterns are not so sensitive as to be affected by small amounts of impurities. Of course, the elimination of known foreign materials from commercial dyes is preferable prior to x-ray examination. The diffraction lines of crystalline materials, such as inorganic salts, would add to the pattern of the dyestuff, thus complicating its identification. Amorphous materials would obscure the pattern with one or more broad diffraction bands (or halos). A special crystallization is seldom necessary as most dyes are in a well crystallized state. If a characteristic pattern is not obtained, owing to poor crystallinity, there is no objection to transforming the dye into a more easily recognizable form by crystallization, heating, melting and quenching, or sublimation. Only 10 to 20 mg. of the sample are required. A glass or plastic capillary can be filled with this material, or the powder can be transformed into a flat or cylindrical tablet by pressing.

Polymorphism. Attention must be given to the polymorphism of organic dyes in considering their x-ray diffraction patterns. The phenomenon whereby a substance may exist in different solid states which are characterized by different space lattices and physical properties is known as polymorphism. The differences such as density, melting point, crystal lattice, color, hardness, etc., disappear on dissolving, melting, or vaporizing the substance. The polymorphic modification or physical isomers must be regarded as distinctive individual forms in the solid state, although possessing the same chemical structure. The various modifications can be observed best by powder patterns because the crystal lattice is changed by polymorphism. It was not known that organic dyes existed in different polymorphic forms until they were examined extensively by x-ray. Polymorphism of organic pigments has also been observed by electron diffraction and electron microscopy. The results of this work have been published by Hamm and Van Norman (*11*).

The x-ray patterns of three polymorphic modifications (alpha, beta, and gamma forms) of metal-free phthalocyanine are shown in Figure 1. This blue dyestuff, whose chemical composition is $C_{32}H_{18}N_8H_2$, is the only organic dyestuff "fingerprinted" to date in the A.S.T.M. card index (*1*). The three strongest lines for the beta form are given as 3.83, 3.45, and 9.64 A. The alpha and gamma forms of the dye are used commercially as excellent pigments, the former well known as Heliogen Blue G or as Monastral Fast Blue G (I.C.I.). Phthalocyanines were investigated by Robertson (*18*) using single crystals of the beta form. This splendid study completely revealed the structure of phthalocyanines. Single crystals of the beta form are easily obtainable by sublimation or crystallization in various high-boiling organic liquids, such as quinoline, nitrobenzene, or trichlorobenzene. In contrast to the alpha and gamma forms, the beta modification exhibits a high tendency to crystallize.

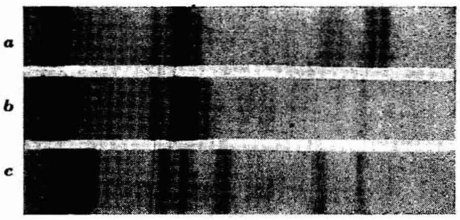

Figure 1. Polymorphic Forms of Metal-Free Phthalocyanine

a. Gamma form
b. Alpha form
c. Beta form

The colors of various polymorphic forms are sometimes very different—e.g., the color of carbon as diamond and graphite, or the scarlet tetragonal and the yellow rhombic forms of mercuric iodide. On the other hand, sometimes the color of different forms is the same or similar—e.g., both rhombic and monoclinic sulfur are yellow; both trigonal and rhombic calcium carbonate (calcite and aragonite) are white.

Among the substances which may change in color as they assume different polymorphic forms are organic dyestuffs. Only those dyes can be used commercially as pigments which remain in the same modification under normal conditions or at least are not seriously affected in color by polymorphic change. If this does occur, the fastness of the dyeing would be affected easily. Polymorphism in organic dyestuffs was observed by x-ray diffraction as early as 1933, when it was found that aniline-azo-2-naphthol, known as Sudan Orange R, existed in four different modifications. These polymorphic forms have no practical significance, because this dyestuff is used for the coloring of fats and waxes in the state of a solid solution. Polymorphic forms are more important, however, for pigments, dyes applied in the solid state for coloration of lacquers, plastics, gum, paper, etc. Here the different physical properties of the various forms result in differences in tinctorial qualities.

Comparison by Standard Samples. In Germany, the diffraction pattern of an unknown sample was identified by comparison with standards. A large collection of x-ray patterns of dyestuffs was available there and these were classified on the basis of their chemical characteristics and tinctorial behavior. Finding the correct standards for comparison presented little difficulty, because enough information as to the structural and functional characteristics of the dye was ascertainable. However, many years are required for the collection of a large set of standard samples, and such is available in only a few places. Wider use of the x-ray method is dependent upon the extensive availability of diffraction data of standard dyestuffs and the adoption of a general classification system.

Hanawalt has suggested classification by determining the spacing values and intensities of the three strongest diffraction lines of the powder pattern. These data make it possible to select easily from a reference book or card index (*1*) several substances whose three strongest lines have the same spacing values as those of the unknown. The sample can then be completely identified by comparing its entire diffraction pattern with these few standards.

Use of this method for dyes requires that the A.S.T.M. card index file be extended to include organic dyestuffs as a special group. Collections have been published in Analytical Chemistry recently for such organic substances as phenyl isocyanate derivatives of some phenols (*16*), hydrazones of some aldehydes and ketones (*6*), anilides of saturated aliphatic acids (*17*), explosives (*19*), penicillins (*5*), and surface-active agents (*2*). The powder patterns of the frequently used commercial dyes listed in the color index should be cataloged first, after which less common and new dyestuffs should be added. Many of these data are undoubtedly known in different places, but are not available to all who are interested in them. Such a collection would be of benefit not only to x-ray workers, physicists, crystallographers, and organic chemists in scientific laboratories, but also to dyestuff manufacturers and dyers (dyestuff users). If international cooperation were obtained, the considerable amount of work required in securing a collection of this kind could be greatly minimized. Inasmuch as excellent equipment is available at present, which makes use of monochromatic x-ray radiation and Geiger counters for registering the intensities of diffraction lines with high accuracy (*5*), it should be possible to secure the required data in a relatively short time.

Limitation of X-Ray Technique. In some cases the crystallites of the substance may be too small or the crystal lattice may be distorted, resulting in a powder pattern whose lines are either diffused or too few in number to give an exact characteristic. The x-ray method cannot be used under such circumstances, nor is it applicable for the few amorphous dyestuffs, such as some sulfur dyes, some complex dyes with chromium, and some mixtures. The crystallinity of commercial dyes in the form in which they are received is sometimes poor, but can be considerably improved by the methods mentioned above.

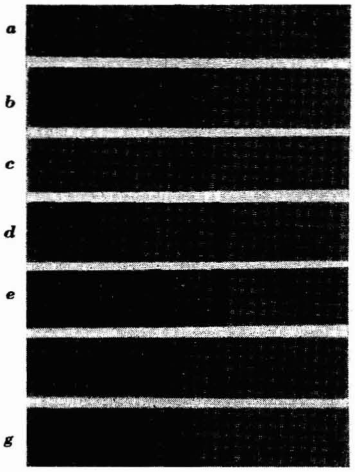

Figure 2. A Series of Azo Dyes

a. Aniline-azo-2-naphthol
b. *o*-Toluidine-azo-2-naphthol
c. *m*-Toluidine-azo-2-naphthol
d. *p*-Toluidine-azo-2-naphthol
e. *m*-Xylidine-azo-2-naphthol
f. *p*-Xylidine-azo-2-naphthol
g. Mesidine-azo-2-naphthol

X-ray diffraction is not sensitive for the determination of impurities or of the minor components in a mixture. Quantitative methods for accomplishing the latter have been proposed, and may be successful in certain cases, especially for minerals or other inorganic materials (*7*). However, when the photographic method is used this technique has not much practical value for dyestuffs. Therefore it is necessary, when dealing with mixtures of dyestuffs, to isolate the compounds first and identify them separately by the x-ray pattern in a nearly pure condition.

To evaluate the effectiveness of the x-ray technique for mixtures, two well crystallized dyestuffs of widely different x-ray diagrams were mixed in known percentages. It was found that the photographic method was incapable of revealing the presence of the minor component of a mixture unless it represented more than 2% of the whole, and even in these cases determinations can be made only in a semiquantitative way. Use of the photometer and other improved techniques may increase considerably both the sensitivity and accuracy of this method, but in most cases will not be of practical value for dyestuff mixtures. The components of a mixture are often formed together, and in this process their crystallinity is altered. Comparison of a poorly crystallized specimen with standard samples made from perfectly crystallized components could also yield entirely false values. Brindley (3) has pointed out that if the absorption of the compounds is not fully taken into account, there is possibility of significant error in the evaluation of the x-ray powder patterns of mixtures, even when comparison is made with standard samples.

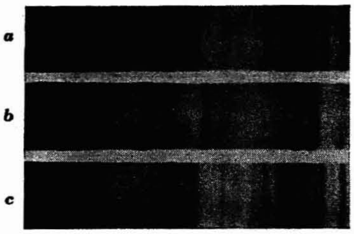

Figure 3. Identification of Setacyl Direct Orange SRR Supra (Geigy)

a. Setacyl Direct Orange SRR Supra (Geigy), melting point 135° C.
b. *p*-Nitroaniline-azo-*o*-toluidide, melting point 136° C.
c. *p*-Nitroaniline-azo-*m*-toluidide, melting point 135° C.

Fortunately, effective methods are known for the separation of the various dyestuffs in a mixture, even when they have similar properties. Insoluble materials may be isolated by fractionated sublimation in high vacuum, and soluble substances by chromatography. For example, even though only 1 gram of Camacyl Seal Brown G (Campbell and Company) was available for test, it became evident after a short study using chromatography and the x-ray technique, that this dyestuff is composed of the following four substances: 4-nitroaniline-azo-*p*-xylidine, 5-nitro-2-anisidine-azodimethylaniline, tetraminoanthraquinone, and a small amount of triaminoanthraquinone.

EXAMPLES OF IDENTIFICATION

Sensitivity of Technique. In Figure 2 are shown the powder patterns of seven azo dyes, some of which are used commercially under the names of Sudan Orange and Ceres Orange. One of the components (2-naphthol) is the same in all samples of the series, while the other component is changed only slightly. The fact that small variations in the structure of the molecule can be easily identified by these patterns demonstrates the sensitivity of the x-ray technique in this respect. It will be noted in samples *b*, *c*, and *d* that the different positions of the CH$_3$ group produce entirely different powder patterns.

The powder pattern of Setacyl Direct Orange SRR Supra (Geigy) is illustrated in Figure 3 as an example of the use of the x-ray technique for dyestuff identification. The certain determination of this commercial dye as either *p*-nitroaniline-azo-*o*-toluidide, *b*, or *p*-nitroaniline-azo-*m*-toluidide, *c*, by the melting point is difficult, because both substances melt at approximately the same temperature. The x-ray patterns show unquestionably that the commercial dye, *a*, is *p*-nitroaniline-azo-*m*-toluidide.

Figure 4 illustrates the identification of Maroon 331 by x-ray diffraction. It had been suspected that this dye probably was made from one of the following compounds: *a*, 3-nitro-4-toluidine-azo-2,3-hydroxynaphthoic acid–*m*-nitroanilide or *b*, 3-nitro-4-toluidine-azo-2,3-hydroxynaphthoic acid–4-nitrotoluidide. The second compound is the same as the first except for the presence of a CH$_3$ group. Differentiation between the two products was impossible either by elementary analysis or by dye tests, because their tinctorial properties are very similar. When heated, both compounds decompose without showing a definite melting point, thus eliminating the use of this method. However, when the diffraction pattern of the commercial sample, *c*, is compared with those of the other two compounds as shown in Figure 4, it becomes immediately evident that Maroon 331 is identical with the first, *a*, and different from the second, *b*.

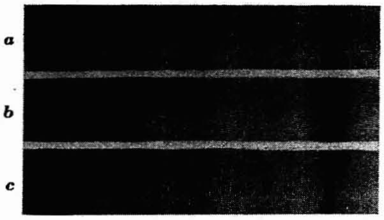

Figure 4. Identification of Maroon 331

a. 3-Nitro-4-toluidine-azo-2,3-hydroxynaphthoic acid-*m*-nitroanilide
b. 3-Nitro-4-toluidine-azo-2,3-hydroxynaphthoic acid-4-nitrotoluidide
c. Maroon 331

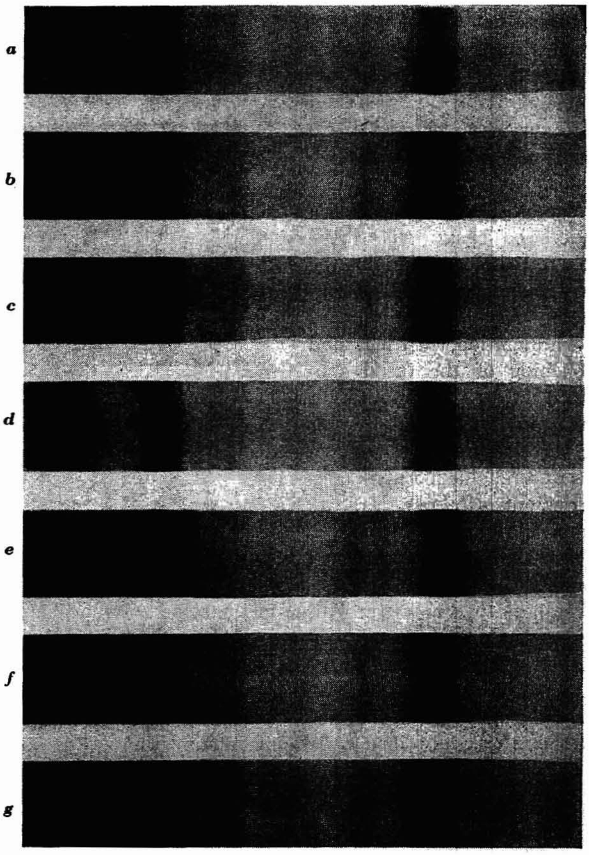

Figure 5. Samples of Indanthrene Brilliant Blue R (I.G.)

a. Beta form
b. Beta (alpha) form
c. Alpha (beta) form
d. Alpha (beta) form
e. Delta form
f. Beta form
g. Alpha (beta) form

In Figure 5 are shown the powder patterns of seven samples of Indanthrene Brillant Blue R (a very pure commercial brand of indanthrone) obtained between 1933 and 1937 at I. G. Farbenindustrie plant at Ludwigshafen, Germany. Some of the samples were taken from the industrial production of the plant. In spite of the standardization practiced by every dye manufacturing company to avoid any variation, these seven diffraction patterns are considerably different. Only samples *a* and *f* are in the beta form (15), whereas samples *b*, *c*, *d*, and *g* contain more or less of the alpha form, and sample *e* is in the delta form. The delta modification has no tinctorial value and none of the more than 35 standard commercial brands of indanthrone manufactured by I. G. in 1938 was in this form.

Detection of Polymorphism. Various samples of the same organic dye are often not entirely identical because they may exist in different polymorphic forms and give different powder patterns. For x-ray identification it is necessary that both the commercial dye and the standard samples be compared in the same polymorphic form. This may be illustrated by the examination of Caledon Jade Green 3BS (I.C.I.). As shown in Figure 6, the powder pattern of this dye as received does not correspond with 12,12′-dimethoxydibenzanthrone, 12,12′-diethoxydibenzanthrone, or 12,12′-dibutoxydibenzanthrone. However, after sublimation in high vacuum the four samples are transformed into a comparable state and it becomes clear from their powder patterns that Caledone Jade Green 3BS is identical with 12,12'-diethoxydibenzanthrone.

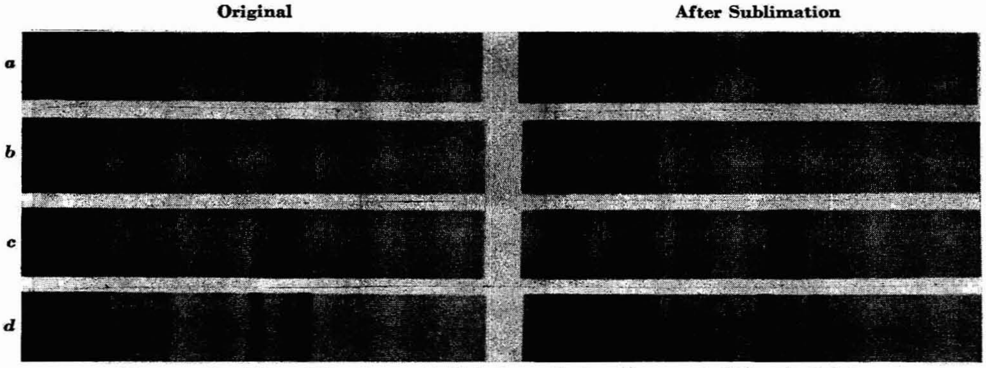

Figure 6. Identification of Caledon Jade Green 3 BS (I.C.I.)

a. Caledon Jade Green 3 BS (I.C.I.)
b. 12,12'-Dimethoxydibenzanthrone
c. 12,12'-Diethoxydibenzanthrone
d. 12,12'-Dibutoxydibenzanthrone

Because samples must be in the same polymorphic form to permit x-ray comparison, it is recommended that all substances be converted to the same form before beginning the x-ray examination. This conversion may be accomplished by sublimation, melting, and quenching of the fused substance, or by redissolving. For dyes soluble in organic liquids the latter is the most convenient and reliable method and is carried out as follows: First, all samples in question should be dissolved to a cold saturated solution in the same liquid. Then a part of this solution should be allowed to evaporate at room temperature. To ensure that the substances thus obtained will be in the same polymorphic form, seed crystals are mutually introduced into the solutions. This method effects no purification of the substance, but it is more reliable than crystallizing from hot saturated solutions (as is normally practiced in organic laboratories) because the samples are thus always obtained in the same modification.

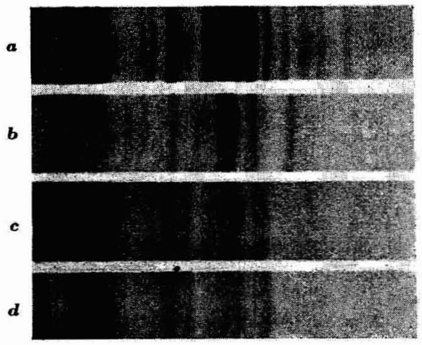

Figure 7. Identification of a Competitive Sample of Zapon Fast Yellow C2G (I.G.)

a. Original competitive sample
b. Original standard sample
c. Competitive sample redissolved in methylene chloride
d. Standard sample redissolved in methylene chloride

For example, the powder pattern of a competitive sample of Zapon Fast Yellow C2G as received, *a*, differed from that of the standard sample, *b*, as shown in Figure 7. A careful chemical investigation showed them to be identical, suggesting that the two specimens existed in different modifications. After redissolving in methylene chloride they changed into a third form *c* and *d*, respectively which was the same for both. Thus the x-ray technique proved that the competitive sample was indeed identical with Zapon Fast Yellow C2G and that this dyestuff was capable of assuming three polymorphic forms.

Solution of Special Problems by X-ray Method. The x-ray method is also very valuable in the field of dyestuff chemistry for the solution of difficult research problems. The investigation of the compounds formed by the action of sodium hydroxide solutions on alizarin illustrates this. It is known that different sodium alizarates are formed by this reaction which have an intense violet or blue color. With one exception—monosodium alizarate—they are not clearly identified by chemical means, as they decompose upon washing or purifying and are destroyed even by the moisture or carbon dioxide of the air. They exist only in the presence of sodium hydroxide of a special concentration. As the diffraction pattern is not sufficiently sensitive to reveal foreign substances, it can be used for the investigation of materials that are not entirely free of impurities. The examination of the different nearly pure reaction products obtained by increasing sodium hydroxide concentrations revealed that beside monosodium alizarate, three disodium alizarates are formed and also two other complex sodium compounds which produce four sodium hydroxide molecules and one alizarate molecule after hydrolysis (Figure 8). This information made it possible to determine the conditions of their formation, stability, and solubility in diluted sodium hydroxide.

The x-ray technique has also been used in investigating the dyestuffs on dyed cotton and rayon fibers. The amount of the dye in normal dyeing is too small to be visible in the diffraction pattern of the sample. However, Valko's method (*20*) of strong dyeing makes it possible to obtain viscose and cuprammonium filaments or films which contain 10 to 50% dyestuff and which are suitable for x-ray identification. As a result of his work in this field, Valko divided vat dyes into three different groups with relation to their crystallinity on the dyed cellulose. The crystallization of the dyestuff, which can be readily recognized by the aid of x-ray diffraction patterns, is responsible for the peculiar behavior of many vat dyes.

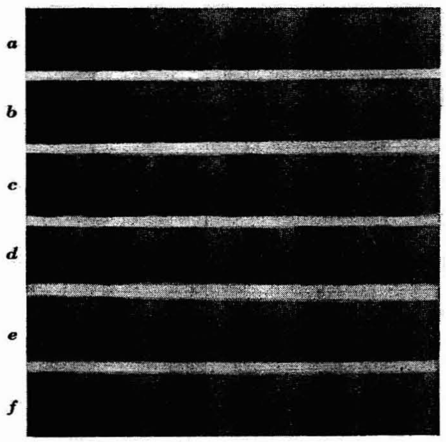

Figure 8. Sodium Alizarates Obtained by Action of Increasing Concentrations of Sodium Hydroxide on Alizarine

a. Monosodium alizarate
b. Blue disodium alizarate
c. Violet disodium alizarate
d. Brown disodium alizarate
e. "Tetrasodium" alizarate I
f. "Tetrasodium" alizarate II

Textile materials dyed with Indanthrene Brilliant Scarlet RK, for example, change from orange to scarlet on soaping or even on rinsing. In Figure 9 is shown a series of diffraction patterns of cuprophane film which was colored by this vat dye. In the diffraction pattern of the dyed material, *b*, no lines are visible except those of the hydrated cellulose as in the undyed film *a*, indicating that the dyestuff is present in an amorphous state. After rinsing, *c*, and even more so after soaping, *d*, the strongest diffraction lines of the dyestuff, *e*, appear as a proof of crystallization, explaining the reason for the color change from orange to scarlet. Similarly, the fact that Caledon Ming Blue BX (I.C.I.) crystallizes on rayon and not on cotton explains why it dyes rayon fabrics violet and cotton fabrics blue. This process is analogous to the well known recrystallization of metals by heating and was first observed on dyed fibers by the microscopic studies of the famous Swiss colorist, R. Haller, who called it "physical condensation" (*9, 10*).

OTHER METHODS OF IDENTIFICATION

Dye Tests and Chemical Investigations. The identification of organic dyes is usually attempted by colorimetric tests and by determination of the chemical composition. Use of physical methods of investigation such as spectrophotometry has increased during recent years. The dyeing methods are empirical in nature and are necessary to determine the tinctorial properties and the technical value of the dyestuffs. Such tests can be conducted conveniently and they reveal the character of the dye in a short time. Chemical examination is more time-consuming. First, the commercial sample must be purified, its solubility must be examined, and its melting point determined, and then an elementary analysis of the dyestuff must be made. The reaction of the dye to heat and its behavior in the presence of acids, bases, oxidative and reductive agents, etc., must be examined. An experienced dyestuff chemist can find out in a relatively short time what compound is in question, provided it is a common dyestuff. In most cases it is advisable to include an x-ray examination for confirmation of the result by an entirely different technique.

Identification by chemical means is much more difficult in dealing with uncommon dyes, new substances of complicated structure, or mixtures of several dyestuffs. In such cases a long investigation is required, including finally a synthesis of the dyestuff in question. Even then it may not be successful, especially when only a small sample of the unknown dye is available. Under such circumstances the x-ray technique is of outstanding value in facilitating examination of the dyestuff. The various substances obtained during the detailed chemical examination (provided they are crystallized) can be identified by x-ray pattern very easily, thus saving considerable time by eliminating the necessity for their individual chemical investigation. This is possible because the x-ray technique is applicable for organic compounds regardless of their color, such as basic materials of dyestuffs, intermediates, or products of decomposition. Sometimes it is preferable as the first step in the identification procedure to compare the x-ray pattern of the unknown dye with those of several others of known chemical composition which are suspected to be identical with the sample in question. However, it is only in rare cases that colorimetric and chemical tests can be omitted entirely.

Heated Specimen Microscopy. In addition to the x-ray technique, mention should also be made of another method of identification of organic materials—namely, examination of heated specimens by the light microscope. This latter technique, practiced in recent years by A. and L. Kofler (*14*), makes it possible to observe at the same time the melting point, sublimation, crystalline form, color, optical properties, polymorphism, formation of mixed crystals, and other characteristics of organic substances. This examination can be done quickly and with a very small amount of the material. Obviously, the results obtained characterize a substance very well. This technique is capable of revealing any structural changes and small percentages of minor components. If corresponding data on known dyestuffs were available, it would provide an excellent "fingerprinting" method. In spite of its usefulness, this method has not been applied to dyestuffs. The method is mentioned here from the standpoint of the development of reliable and sensitive methods for identifying complicated organic compounds in a short time using small specimens. Like the x-ray technique, heated specimen microscopy may have its limitations and disadvantages as a method of solving this problem, but both are beyond any question of outstanding value. Their extensive use is dependent, however, upon the availability of a large collection of comparative data.

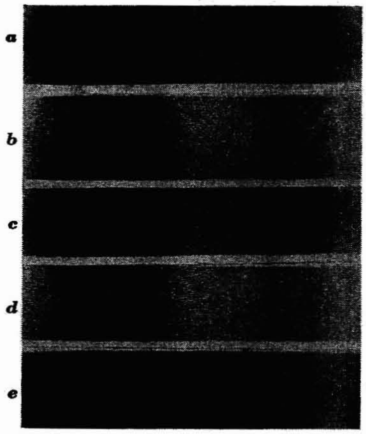

Figure 9. Recrystallization of Indanthrene Brilliant Scarlet RK (I.G.) Dyed on Cuprophane Film

***a*. Cuprophane film undyed. Equatorial diffraction lines $A_0(101)$, $A_3(10\bar{1})$, and $A_4(002)$ of hydrate cellulose are visible**
***b*. Cuprophane film dyed with 14.4% Indanthrene Scarlet RK after oxidation. Only diffraction lines of hydrate cellulose are visible. Dyestuff is in amorphous state. Color, orange**
***c*. Same as *b* after 24 hours' rinsing. Besides diffraction lines of hydrate cellulose, strongest lines of dye begin to be visible. Color, scarlet**
***d*. Same as *b* after soaping 1 hour at 100° C. Same pattern as *c*, but more clearly visible. Color, scarlet**
***e*. Commercial sample of Indanthrene Brilliant Scarlet RK in powder form**

SUMMARY

The x-ray technique for the identification of organic dyes has been used effectively in Germany since 1933 in conjunction with conventional chemical investigations and dye tests. It is a very simple method of identification and is capable of a high degree of sensitivity. The extensive application of this technique is dependent upon the collection, classification, and publication of diffraction data on organic dyestuffs for use as standards of comparison. Identification by x-ray is particularly valuable when only small samples are available for examination, when dealing with products which do not show a definite melting point, when the unknown substance melts at approximately the same temperature as other dyestuffs with which it may be identical, and when the determination of the mixed melting point fails to give a conclusive result.

ACKNOWLEDGMENT

The development of the identification of dyestuffs by x-ray diffraction patterns is partly due to the interest shown in this technique by Alfred Kirsch and Hans Krzikalla. Dr. Kirsch was charged with the responsibility of identifying complicated competitive dyes of commercial value and frequently had difficulties in doing so by the conventional methods. The author is indebted to him for many helpful discussions on the subject. The outstanding interest in this work of the late Arthur Lüttringhaus and Max A. Kunz, former chiefs of the main laboratory of the I. G. Farbenindustrie plant at Ludwigshafen, must also be gratefully acknowledged.

The excellent work of Jack Salmon and his assistants in reproducing the x-ray diagrams shown in this report is gratefully acknowledged. Although the diagrams are not perfect, they represent the best possible reproductions from the pictures available at present. The help of Norman E. Roberts, information and editorial specialist, Textile and Leather Products Section, Office of the Quartermaster General, in the writing of this paper is appreciated greatly by the author.

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Spectrophotometric Determination of Nicotine

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A rapid and accurate method is presented for determination of nicotine by ultraviolet spectrophotometry. The value of the method is illustrated by comparing analyses of a wide variety of tobacco and nicotine samples by this method and the gravimetric silicotungstic acid method. Neither method differentiates between nicotine and nornicotine.

DURING a study seeking richer natural sources of nicotine and improved methods for its recovery from tobacco, a rapid reliable method was required for determination of nicotine in samples of many different types.

The silicotungstic acid method of the Association of Official Agricultural Chemists, based on a procedure proposed by Chapin (*4*) as a modification of Bertrand's (*2*) method, yielded accurate results for most samples. The method, however, is extremely slow; it requires 24 to 48 hours. Moreover, as shown by Ogg *et al.* (*9*), it is subject to serious errors when large amounts of ammonia or ammonium salts are present. Another defect, usually not serious, is that it does not distinguish nornicotine from nicotine.

The methods of Kissling (*7*), Toth (*12*), and Garner (*5*) are based on the titration of nicotine after isolation of the free base by distillation, extraction, or a combination of these procedures. Because these methods measure all basic material in the test solution as nicotine, they lead to erroneous results in the presence of other bases, which are difficult to separate from nicotine. The colorimetric method proposed by Markwood (*8*), depending on formation of the nicotine 2-naphthylamine–cyanogen bromide complex, is relatively rapid but is not specific for nicotine, because it is subject to interferences by a large number of other substances (*13*). The dipicrate method (*10*) is subject to possible error because of the solubility of the picrate salt.

Other investigators have attempted to shorten the time for analysis of nicotine in tobacco and similar materials by employing micro and semimicro distillation techniques (*1*, *6*). These methods, however, magnify sampling errors, and difficulties caused by frothing are encountered in using the Avens apparatus.

BASIS FOR ULTRAVIOLET ABSORPTION METHOD

The work of Swain *et al.* (*11*) on the ultraviolet absorption of nicotine and related compounds suggested that direct ultraviolet spectrophotometry might be applicable to the quantitative determination of nicotine. The ultraviolet absorption spectrum of nicotine is characterized by a moderately strong, sharp absorption maximum near 260 mμ. The exact position and intensity of the maximum are influenced by the nature of the solvent, and the intensity is markedly affected by acid (Figure 1). Thus the specific extinction coefficient and wave length of the absorption maximum are 18.6 at 260 mμ in water, and 34.3 at 259 mμ in acidified water. (The value 34.3 is an average of five separate weights of a sample of purified nicotine.) Because sensitivity of detection is greater in the latter medium, direct ultraviolet spectrophotometry is admirably suited to determination of nicotine in the acidified aqueous media in which it is commonly obtained or isolated in the laboratory. The applicability of the method to the determination of nicotine in a variety of test solutions was therefore investigated.

The acid concentration is not critical, provided it exceeds 0.02 *N*. Solutions used in this investigation were acidified with hydrochloric acid to 0.05 *N*. Acids such as sulfuric or phosphoric are equally suitable. Spectral densities of solutions, measured on a Beckman Model DU spectrophotometer, were confined to the range 0.2 to 0.8 by adjustment of concentration, or in extremely dilute solutions by using cell lengths up to 5 cm. The acidified nicotine solutions obeyed Beer's law over the range tested, 1 to 30 mg. per liter.

Interferences to be expected in this method are of two types: structurally related alkaloids containing the pyridine chromophore, and unidentified constituents absorbing in this region of the spectrum, either of which may accompany nicotine in the process used for its isolation.

Nornicotine, if present, will be determined as nicotine, because it has the same chromophore and a spectrum indistinguishable from that of nicotine (*11*). The error is somewhat enhanced by the fact that the specific extinction coefficient of nornicotine is

10% higher than that of nicotine. Other methods for determination of nicotine are subject to the same limitation. For most samples, however, and especially those from which the nicotine has been separated by steam distillation, the positive error contributed by nornicotine is usually unimportant, as nornicotine is present in most tobaccos only in small proportion.

Interferences of the second type may include substances such as alkaloid degradation products, plant pigments, and lipides. Extraneous absorbing constituents are more abundant in nicotine solutions obtained by extraction than by distillation procedures. The absorption spectra of a number of typical solutions obtained by both procedures were examined. By comparing these spectra with that of pure nicotine, it was found that the extraneous "background" absorption underlying the nicotine absorption in the spectra of the sample was essentially linear from 230 to 290 mμ. It therefore became a simple matter to correct for this extraneous absorption.

The background correction (*3*) as applied here involves measurement of the spectral densities at 259 mμ (maximum) and at wave lengths 23 mμ on each side of the maximum—that is, at 236 mμ (near minimum) and 282 mμ. For pure nicotine in acidified water, the relationship between the observed densities at these wave lengths is $D_{259} = 1.059\ [D_{259} - {}^1/_2(D_{236} + D_{282})]$. If a solution contains, in addition to nicotine, extraneous substances having linear absorption characteristics (regardless of slope) between 236 and 282 mμ, it can be shown that $D'_{259} = 1.059\ [D_{259} - {}^1/_2(D_{236} + D_{282})]$, where D_{259}, D_{236}, and D_{282} are the observed densities and D'_{259} is the density corrected for background absorption—that is, the density contributed by the nicotine in the solution. The difference between observed and corrected densities, $D_{259} - D'_{259}$, is a measure of the extraneous absorption. The magnitude of corrections in the wide variety of samples studied here ranged from 0 to 5%. However, this correction is valid only when the background absorption is linear or nearly linear from 236 to 282 mμ.

PROCEDURE

An aqueous solution containing the alkaloid and having an acid concentration equivalent to approximately 0.05 *N* hydrochloric acid is ordinarily prepared from a source such as the following: a commercial or laboratory preparation of nicotine or nicotine concentrate; an acidified water extract of a solution of nicotine in solvents such as kerosene and gasoline; or a steam distillate, collected in dilute acid, of a material containing nicotine. The acidified solution, representing a known sample weight, is made to volume and diluted with acidified water, if necessary, until spectral densities observed at wave lengths 236 and 259 mμ lie within the optimum range 0.2 to 0.8. Acidified distilled water is used as a blank for the density measurements. Dilutions are simplified if a sample containing 10 to 20 mg. of nicotine is used and the original volume is made to 1 liter. The observed density in a 1-cm. cell at 236 mμ will then be in the specified range. The density at 282 mμ need not be measured with high accuracy and can therefore be determined on this same solution. The density at the maximum, 259 mμ, is about 10 times that at 236 mμ, and in general will require a tenfold dilution of the solution in order to obtain a value within the specified range.

The density D'_{259}, corrected for background absorption and referred to the original volume, *V*, which was used in the determination of D_{236}, is calculated by the equation

$$D'_{259} = 1.059\ [FD_{259} - {}^1/_2(D_{236} + D_{282})]$$

where D_{236} and D_{282} are the densities observed at 236 and 282 mμ in *V*, and D_{259} is the observed density in the diluted solution (dilution factor *F*, usually 10).

The concentration of nicotine in *V*, in grams per liter, is then

$$c = D'_{259}/34.3\ b$$

where 34.3 is the specific extinction coefficient (defined by $k = D/bc$), or spectral density referred to cell depth of 1 cm. and concentration of 1 gram per liter of pure nicotine in acidified water at 259 mμ; and *b* is the inside depth of the cell in centimeters. The depth of the cell is usually 1 cm., but depths up to 5 cm. are used for analyses of solutions low in nicotine.

The total weight of nicotine in the sample, in grams, is simply the concentration, *c*, times the original volume, *V*, which is usually 1 liter.

The spectrophotometric method permits the estimation of nicotine in an acidified aqueous solution in 10 to 15 minutes, depending on the number of dilutions necessary.

COMPARISON OF SPECTROPHOTOMETRIC AND A.O.A.C. METHODS

To compare the reliability, precision, and accuracy of the spectrophotometric method for nicotine with that of the official A.O.A.C. silicotungstate method, ten successive analyses of each of two solutions containing different concentrations of nicotine were made by each method (Tables I and II). The nicotine concentrations of the two solutions were near the limits specified by the A.O.A.C. method. For the solution of high nicotine concentration, which contained approximately 100 mg. of nicotine per 100 ml., the standard deviation of the results of the spectrophotometric analyses was 0.144, as compared with 0.089 for the results

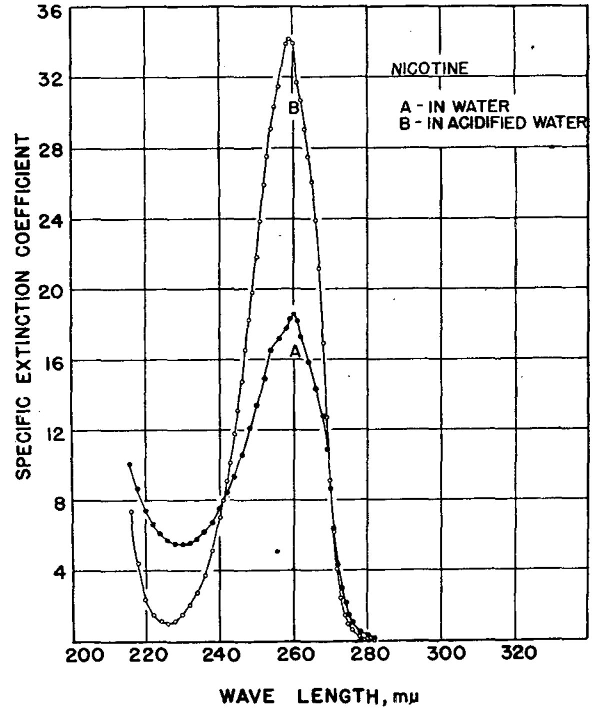

Figure 1. Ultraviolet Absorption Spectrum of Nicotine

A. In water
B. In acidified water, 0.05 *N* HCl

Table I. Determination of Nicotine in Solutions High in Nicotine

	Nicotine Found, Mg. per 100 Ml.		
	Spectrophotometric		Chemical
	102.8		101.3
	102.8		101.5
	102.8		101.3
	102.8		101.3
	103.0		101.3
	102.5		101.2
	102.5		101.2
	102.7		101.4
	102.8		101.2
	102.8		101.3
$\overline{X}$	102.75	$\overline{X}'$	101.30
S	0.144	S	0.089

F observed = 2.58
10% level, *F* = 3.18
2% level, *F* = 5.35

Table II. Determination of Nicotine in Solutions Low in Nicotine

	Nicotine Found, Mg. per 100 Ml. Spectrophotometric		Chemical
	10.57		10.30
	10.60		10.22
	10.56		10.24
	10.60		10.08
	10.56		10.14
	10.60		10.18
	10.55		10.26
	10.57		10.29
	10.62		10.27
	10.61		
$\bar{X}$	10.584	$\bar{X}'$	10.220
S	0.021	S	0.071

F observed = 11.0
2% level F = 5.91

obtained by the A.O.A.C. chemical method. The F value limit indicates that both methods are equally reliable. For the solution of low nicotine content, which contained approximately 10 mg. of nicotine per 100 ml., the results of the analyses show a standard deviation of 0.021 mg. for the spectrophotometric method and 0.071 mg. for the chemical method, indicating a greater precision for the spectrophotometric method. The observed F value of 11.0 when compared with 5.91, the F value for the 2% level, indicates greater precision for the spectrophotometric method for solutions low in nicotine.

The effects of ammonium and alkali salts on the analysis of nicotine by the spectrophotometric method were investigated, because the work of Ogg *et al.* (*9*) had demonstrated that the silicotungstic acid method is subject to considerable error when these salts are present, especially when the nicotine concentration is low. The two basic nicotine solutions used for these tests contained approximately 10 and 80 mg. of nicotine per 100 ml. of solution, representing the approximate limits of nicotine concentration recommended for analysis by the A.O.A.C. method. Nicotine-salt solutions were prepared by adding 10% ammonium chloride, ammonium sulfate, and sodium sulfate, respectively, to the two basic nicotine solutions. High salt concentrations were chosen to exaggerate the effect.

Table III. Effects of Ammonium and Sodium Salts on Determination of Nicotine in Solutions

Salt	Concentration %	Low Nicotine Spectrophotometric	Low Nicotine Chemical	High Nicotine Spectrophotometric	High Nicotine Chemical
		Mg./100 Ml.			
None	0	10.67	10.59 10.53	82.2	81.3
Ammonium chloride	10	10.61	7.29 10.41	81.9	91.7 94.0
Ammonium sulfate	10	10.61	6.44 6.27	82.1	88.1 85.7
Sodium sulfate	10	10.61	8.22 6.27	82.4	81.3 81.2

Table IV. Distillation of Nicotine from Modified Griffith Apparatus

Time, Min.	Slow Distillation Rate: Volume of distillate, ml.	Slow: Nicotine found, %	Slow: % of total nicotine	Rapid Distillation Rate: Volume of distillate, ml.	Rapid: Nicotine found, %	Rapid: % of total nicotine
Still Charged with Dried Leaf Tobacco Ground to Pass 100-Mesh						
0	0.0	0.0	0.0	0	0.0	0.0
2	0.1	0.0	0.0	..	..	...
4	56.0	8.01	94.9	..	..	...
6	96.2	8.12	96.3	184	8.16	96.7
8	145.4	8.15	96.5	344	8.27	98.0
10	202.4	8.21	97.3	420	8.27	98.0
12	242.2	8.28	98.1	489	8.28	98.1
14	...	..	..	558	8.34	98.9
16	472.3	8.32	98.6	751	8.35	98.9
20	569.3	8.35	98.9	..	..	...
30	854	8.40	99.6	1410	8.38	99.3
60	1852	8.40	99.6	2918	8.46	100.2
Still Charged with Aqueous Nicotine Solution						
					Mg./100 Ml.	
0				0	0	
10				410	10.53	
24				805	10.59	
				Original undistilled solution	10.58	

Table V. Determination of Nicotine in Comminuted Green Tobacco Leaves

Distillation Apparatus		Nicotine Found, % Spectrophotometric %	Chemical %
Modified Griffith		8.08	8.16
		8.13	8.15
		8.21	8.21
	$\bar{X}$	8.14	8.17
A.O.A.C.		8.13	8.23
		8.11	8.16
		8.13	8.16
	$\bar{X}$	8.12	8.18

Table VI. Determination of Nicotine in Dried Tobacco Leaves

Sample	Nicotine Found: Spectrophotometric %	Spectrophotometric Av. %	Chemical %	Chemical Av. %
1-a 1-b	0.85 0.85	0.85	0.85 0.84	0.85
2-a	2.20	2.20	2.21	2.21
3-a	2.46	2.46	2.48	2.48
4-a	3.17	3.17	3.22	3.22
5-a 5-b	4.04 4.09	4.07	4.08 4.16	4.12
6-a 6-b	5.24 5.32	5.28	5.35 5.45	5.40
7-a 7-b	6.25 6.26	6.26	6.44 6.33	6.39
8-a 8-b	8.10 8.09	8.10	8.05 8.05	8.05

Table III shows the results of the analyses of these solutions by the spectrophotometric and chemical methods. It is apparent from these data that the chemical method yields erroneous results when applied to solutions containing salts of the type shown (*9*). The results are low and inconsistent for the solutions of low nicotine content in the presence of salts. They are high and inconsistent for the solutions of high nicotine content in the presence of salts, except for the solution containing the sodium salt. On the other hand, the spectrophotometric method yields results in excellent agreement for all cases, showing no effect from the dissolved salts.

APPLICATION OF METHOD

The spectrophotometric method has been applied to analyses of a wide variety of tobacco materials, including tobacco leaves, tobacco juice, distillates, tobacco juice extracts, and tobacco ensilage made of the whole plant. All samples were simultaneously analyzed by the official A.O.A.C. silicotungstic acid method.

The aqueous solution of the nicotine from each of these materials was prepared by steam distillation from solutions made strongly alkaline with either barium or sodium hydroxide. The still used was a modification of that of Griffith and Jeffrey (*6*). A test for completeness of distillation of the nicotine by a qualitative silicotungstic turbidimetric method indicated apparent complete recovery after 4 to 6 minutes, whereas the spectrophotometric method showed an actual recovery of only 95 to 96%. A 30-minute distillation time was adopted for the regular procedure. This provides adequate time to complete the spectrophotometric analysis and to operate two stills simultaneously.

A test of the rate of distillation of nicotine from an aqueous solution (in Table IV) showed that complete recovery was attained in less than 10 minutes. The longer time required for the distillation of nicotine from dried plant tissue must therefore be due to the time required for nicotine to diffuse through the tissue cells.

Nicotine was determined in green tobacco leaves by the spectrophotometric and silicotungstic acid methods with nicotine distillates obtained from them with both the modified Griffith and A.O.A.C. steam distillation apparatus. Green leaves were chosen for this comparison because they are difficult to sample and to analyze. A laboratory sample was prepared by comminuting the leaves in a Waring Blendor with acidified water. Aliquots were removed for the nicotine analyses and for moisture determination. The results (Table V) indicate no significant difference between the two methods of distillation or methods of analysis. Table VI shows the excellent agreement obtained between the results of the spectrophotometric and chemical methods on samples of dried tobacco leaves ranging in nicotine content from less than 1 to more than 8%.

Table VII shows the results of analyses of tobacco juice, distillates, residues, kerosene extracts, and extract residues, and distillates of ensilaged whole tobacco plants. In all cases there was excellent agreement between the spectrophotometric and chemical methods.

Table VII. Determination of Nicotine in Tobacco Products

Material	Nicotine Found, %	
	Spectrophotometric method	Chemical method
	%	%
Tobacco juice	0.52	0.52
Distillate from tobacco juice	0.59	0.58
Residue in still pot	0.20	0.18
Distillate containing ammonia	0.18	0.17
Distillate from potassium chloride solution	0.58	0.58
Juice stripped with kerosene	0.20	0.20
Kerosene extract	0.34	0.34
Whole plants		
Ensilage (with phosphoric acid)	0.50	0.51
Ensilage (with lime)	0.40	0.38
Ensilage (with sodium chloride)	0.32	0.32
Ensilage (with potassium chloride)	0.37	0.36

SUMMARY

A rapid, sensitive method of nicotine analysis is proposed which gives results concordant with the official A.O.A.C. silicotungstic acid method. Nornicotine, if present, is determined as nicotine by both methods. The spectrophotometric method is unaffected by ammonia or alkali salts in the test solution.

When the method is used in conjunction with a modified Griffith and Jeffrey nicotine distillation apparatus, the time required to determine nicotine by the spectrophotometric method is only 20 to 30 minutes, as compared with 24 to 48 hours required by the A.O.A.C. silicotungstic acid method.

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THE RARE EARTHS

Spectrophotometric Estimation of Certain Rare Earth Elements

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Although the quantitative determination of one rare earth element in a mixture containing others is of considerable importance, the striking similarities in properties which characterize these elements in a given state of oxidation have precluded the development of purely chemical procedures except in those cases—e.g., cerium, europium—where changes in oxidation state may be effected. Physical or physicochemical approaches are, therefore, essential. Methods involving determination of average atomic weight or magnetic susceptibility are ordinarily limited to binary mixtures and are of doubtful accuracy. However, procedures that depend upon evaluation of arc or spark spectra, upon measurement of absorption spectra, or upon neutron absorption are more widely applicable and do not involve material alteration or destruction of the sample.

Of these, procedures involving absorption spectra are the most widely adaptable and probably the most generally useful because of ease of measurement through readily available and convenient instrumentation. The absorption spectra of many of the tripositive rare earth ions are characterized by complex system of comparatively sharp bands at very definite wave lengths in the ultraviolet, visible, and near infrared. Many of these bands in the visible have been used for many years for the qualitative detection and semiquantitative estimation of certain of the elements, but really reliable quantitative procedures involving them have been developed only within the past few years.

Use of absorption spectra for quantitative determinations of the rare earth elements has been reviewed very completely by Rodden (*19*). Briefly, early procedures involved dilution until a given band showed the same intensity, when viewed with a direct vision spectroscope, as given by a standard (*1, 3*); dilution until a given band disappeared (*4, 25*); relation of band width to length of absorption path through use of standards (*2*); and application of spectrophotometric technique (*13*). Partridge and Rodden (*15*) employed a filter photometer for the successful determination of neodymium in admixture with praseodymium and samarium, but were unable to obtain satisfactory results for the latter two elements.

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In two important papers, Rodden (*19, 20*) summarized absorption spectra measured with a Coleman Model 10S spectrophotometer for nitrate solutions of the majority of the tripositive rare earth elements over the range 3500 to 10,000 A. Beer's law was found to apply reasonably well over broad concentration ranges, and both the method and suitable wave lengths for the quantitative estimation of a number of the rare earths in mixtures were outlined. Rodden's general procedure has since been used by a number of other workers (*6, 7, 9-11, 23, 26*) and has proved both rapid and generally accurate.

In conjunction with the general investigations being carried out in this laboratory, rapid determinations of individual rare earth elements in complex mixtures became necessary. Although Rodden's method, as published, was generally useful, it was apparent that complications due to more complete resolution of absorption peaks by the more refined instrumentation at the authors' disposal, limitations of spectral range in Rodden's original studies, and incomplete knowledge of the effects of various other ions upon absorption intensities all indicated the need for further fundamental studies. Accordingly, the absorption spectra of some eleven of the tripositive rare earth ions, scandium, yttrium, and thorium were investigated over the spectral range 2200 to 10,000 A., using chloride, nitrate, acetate, and in many cases perchlorate solutions. In establishing an analytical procedure based upon these spectra, choice of bands free from interferences, adherence of absorption to Beer's law, effects of slit width on band resolution, and effects of added anions were all considered. The present paper summarizes these results and outlines an optimum spectrophotometric procedure which extends and complements that of Rodden.

APPARATUS AND MATERIALS

All spectrophotometric measurements were made with a Beckman quartz spectrophotometer, Model DU, using fused silica cells with an optical light path of 1.000 ± 0.005 cm. This instrument is constructed so that the slit width can be varied from 0.01 to 2.0 mm., giving a nominal band width ranging from 2 to 40 A., depending on the wave length used. At any given wave length, this nominal band width can be varied by altering the sensitivity of the electrical system of the instrument. The only modification made was to cement a slide rule indicator glass (black line up) above the wave-length scale to decrease parallax and increase the accuracy of the wave-length settings.

The wave-length scale was calibrated frequently against the 5460.7 A. emission line from a mercury vapor lamp, as recommended by the manufacturer.

A tungsten lamp was employed for readings above 3200 A. and a hydrogen discharge lamp for readings at lower wave lengths. The silica cells were thoroughly cleaned with nitric acid before each use. They matched exactly in the visible range of the spectrum, but because they did not have the same transmission characteristics in the ultraviolet, cell corrections were determined and applied for readings in this region.

Purified rare earth materials were obtained for the most part from the stocks of the University of Illinois. The europium material used was a pure sample kindly provided some years ago by H. N. McCoy. The praseodymium material was an analyzed sample obtained through the courtesy of the Lindsay Light and Chemical Company. The erbium material was a sample that was purified by the ferrocyanide procedure (*11*). Because of the lack of a completely independent check, its exact purity was unknown. The value given is based upon comparison with other samples of known compositions. The compositions and sources of the materials used are summarized in Table I. Other chemicals were of analytical reagent quality or better.

Table I. Compositions and Sources of Materials Employed

Material	Inventory No.	Composition	Source
$K_3Sc(SO_4)_3$	....	Spectroscopic standard	Adam Hilger, Ltd.
Y_2O_3	YT-15	Atomic weight 88.7	Illinois stock (*5*)
$Th(NO_3)_4$	....	Free from rare earths	Lindsay Light and Chemical Co.
La_2O_3	LA-40	Atomic weight purity	Lindsay Light and Chemical Co.
$Ce(NO_3)_4.2NH_4NO_3$	....	Analytical reagent quality	G. Frederick Smith Chemical Co.
Pr_6O_{11}	PR-L1	93% Pr_6O_{11}, 2% Nd_2O_3, 5% La_2O_3	Lindsay Light and Chemical Co.
Nd_2O_3	ND-33	Free from other rare earths	Illinois stock
Sm_2O_3	SM-35	Free from other rare earths	Illinois stock (*22*)
Eu_2O_3	....	Atomic weight purity	H. N. McCoy (*8*)
Gd_2O_3	GD-5	Atomic weight purity	Illinois stock (*14*)
Er_2O_3	ER-1-2S	98% Er_2O_3, 1% Ho_2O_3 and Tm_2O_3, balance Y_2O_3	Illinois stock
Tm_2O_3	TM-5-R3	88% Tm_2O_3, 11% Lu_2O_3, 1% Yb_2O_3	Illinois stock (*10*)
Yb_2O_3	YB-1a	Atomic weight purity	Illinois stock (*16*)
Lu_2O_3	LU-4R-11	95% Lu_2O_3, 4.7% Tm_2O_3, 0.3% Yb_2O_3	Illinois stock (*10*)

ABSORPTION SPECTRA

Chloride, nitrate, and acetate solutions were prepared by slurrying weighed samples of freshly ignited rare earth metal oxides with water, adding the desired acid in slight excess, and warming until dissolution occurred. The solutions were then evaporated to dryness either on a steam bath or with an infrared heater, the residues were taken up in water, and the process was repeated to remove excess acid. The final, acid-free solutions were diluted to such volumes that concentrations equivalent to 25 grams of rare earth metal per liter were obtained. Cerium (III) chloride was prepared by treating a water solution of cerium (IV) ammonium nitrate with concentrated hydrochloric acid and evaporating to dryness. Dissolution of the residue in concentrated hydrochloric acid and evaporation, repeated twice, gave a white, crystalline product. This material was dissolved in water (with the aid of 2 drops of concentrated hydrochloric acid per 100-ml. volume) to a solution containing 0.1276 gram of cerium per liter. Yttrium chloride solution was prepared from the ignited oxide by the procedure used for the rare earth elements. Scandium and thorium chloride solutions were obtained in the same fashion, except that the freshly precipitated hydrous hydroxides were used.

Perchlorate solutions were prepared by slurrying the weighed oxides with water, heating, and adding the calculated quantity of 10% perchloric acid in small increments; a few minutes were allowed to elapse after each addition to permit the slow reactions to occur. Excess of the rather difficult to remove acid was thus avoided. Concentrations were again equivalent to 25 grams of metal per liter.

Absorption spectra of these solutions were measured against distilled water as a comparison blank over the range 2200 to 10,000 A. Readings were made every 20 A. through the ultraviolet and up to 6000 A. and every 50 A. from 6000 to 10,000 A. Where absorption bands were found, readings were made at narrower wave-length intervals to establish the wave lengths of peak absorptions as precisely as permitted by the instrument. Where the original solution was too concentrated to give accurate log I_0/I values, it was diluted to the necessary concentration. No attempt was made to control temperature accurately, for measurements showed the effects of small temperature variation to be too small to be of importance. The temperatures during readings ranged from 25° to 34° C.

Absorption curves were then plotted as wave length (λ) in Angstroms versus k, the absorption coefficient, where k is defined by the Beer-Lambert expression as

$$k = \frac{\log I_0/I}{cl}$$

In this expression, c is concentration in grams of metal per liter, l the light path (= 1 cm.), and I_0 and I are the intensities of the incident and transmitted light, respectively. Expression of c in moles per liter would give corresponding molecular absorption coefficients (ϵ).

Spectra for chloride, nitrate, acetate, and perchlorate solutions were essentially similar, although the nitrate absorption band in the region of 3000 A. obscured any rare earth bands at wave lengths below 3400 A. The positions of absorption bands in chloride, nitrate, and perchlorate solutions of a given cation were nearly identical. However, in acetate solutions of neo-

Absorption spectra have been investigated over the range 2200 to 10,000 A. for eleven of the tripositive rare earth elements, scandium, yttrium, and thorium, in aqueous solutions of the chlorides, nitrates, acetates, and perchlorates. Specific data are presented for the chlorides (and erbium perchlorate) of those ions which show absorption in the ultraviolet, visible, or near infrared. Data are also given for the effects of slit width on band resolution, for the effects of concentration upon absorption, for wave lengths of bands free from interferences by other rare earth metal ions, for sensitivities of detection at these wave lengths, and for the effects of added ions. Based upon these values, a spectrophotometric procedure adaptable to the estimation of praseodymium, neodymium, samarium, europium, thulium, and ytterbium to within ±1% is outlined. Information relative to the estimation of gadolinium and erbium, where larger errors are encountered, is also presented.

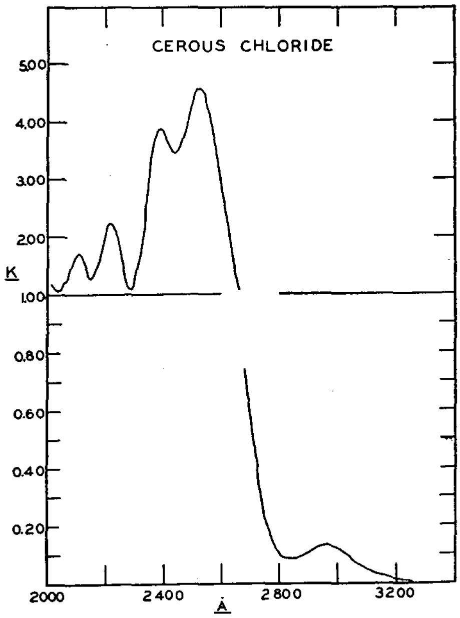

Figure 1. Absorption Spectrum of Cerium(III) Chloride Solution

dymium, samarium, europium, and gadolinium, certain bands were displaced to longer wave lengths. Spectra for chloride and perchlorate solutions agreed in all cases where measurements were made, except with erbium. Erbium chloride solutions did not give reproducible absorption values, although erbium perchlorate solutions gave the same results even after standing for several months.

Because of the greater utilities of chloride solutions and the simplicity of handling them, only the spectra of such solutions, plus that of erbium perchlorate, are included in Figures 1 to 9. Lack of purified samples of terbium, dysprosium, and holmium precluded measurement of their spectra. Scandium, yttrium, lanthanum, lutecium, and thorium ions showed no absorption over the range studied and are, therefore, omitted. For the other materials, only the regions of absorption are included. If, therefore, the complete spectrum is not given—e.g., for cerium chloride, ytterbium chloride—it is understood that the omitted portion contains no absorption bands. The spectrum of praseodymium chloride has been corrected for the neodymium absorption shown by the samples.

The spectra given in Figures 1 to 9 are in excellent agreement with those obtained by Prandtl and Scheiner (*17*) by photographic means. Certain of the weaker bands reported by these authors do not appear in the present data because of the inability of the Beckman instrument to detect them. Excellent agreement is also noted between the authors' spectra and those given over more limited ranges by Rodden (*19, 20*) and those measured over essentially the same range by Kremers (*7*). Spectra given by Stewart (*24*) for perchlorate solutions of the cerium earths are also in excellent accord with the authors' observations.

SELECTION OF ABSORPTION BANDS SUITABLE FOR SPECTROPHOTOMETRIC ANALYSIS

From the analytical point of view, selection of bands free from interference by other rare earth metal ions would be desirable. It is also important that the bands chosen be the strongest in the given spectrum, in order that maximum sensitivity be obtained. Examination of the spectra given in Figures 1 to 9 shows that many of the strongest bands are subject to greater or lesser interference by other rare earth metal ions. In most cases, however, the interfering ions may be determined with accuracy and corrections applied. Such corrections become important only when the interfering ion is present in larger concentration than the ion being determined.

Table II. Spectrophotometric Data

Salt Used	Wave Length	Molecular Absorption Coefficient	Absorption Coefficient	Slit Width	Minimum Sensitivity, 1-Cm. Cell
	A.	ϵ	*k*	*Mm.*	*G. metal/ 100 ml.*
$PrCl_3$	4445	9.85	0.0700	0.025	0.143
$Pr(NO_3)_3$	4445	9.20	0.0654	0.025	0.156
$NdCl_3$	5218	4.33	0.0300	0.013	0.333
$Nd(ClO_4)_3$	5218	4.30	0.0298	0.013	0.336
$Nd(NO_3)_3$	5218	3.88	0.0269	0.013	0.372
$Nd(C_2H_3O_2)_3$	5230	3.80	0.0263	0.013	0.380
$SmCl_3$	4020	3.14	0.0210	0.044	0.480
$Sm(NO_3)_3$	4020	3.28	0.0218	0.044	0.458
$Sm(C_2H_3O_2)_3$	4025	3.68	0.0244	0.044	0.410
$EuCl_3$	3939	2.92	0.0192	0.029	0.520
$Eu(NO_3)_3$	3945	2.05	0.0135	0.053	0.743
$Eu(C_2H_3O_2)_3$	3947	1.92	0.0126	0.053	0.793
$GdCl_3$	2728	2.34	0.0149	0.205	0.670
$Gd(C_2H_3O_2)_3$	2729	3.28	0.0209	0.205	0.479
$Er(ClO_4)_3$	3792	4.00	0.0239	0.030	0.419
$TmCl_3$	6825	2.58	0.0152	0.018	0.659
$Tm(ClO_4)_3$	6825	2.49	0.0147	0.018	0.680
$YbCl_3$	9750	1.94	0.0111	0.026	0.901
$Yb(NO_3)_3$	9750	1.91	0.0110	0.026	0.912
$Yb(ClO_4)_3$	9750	1.96	0.0113	0.026	0.885
$Yb(C_2H_3O_2)_2$	9720	1.99	0.0116	0.026	0.864

The wave lengths summarized in Table II are those which are most useful for analytical determinations. Because cerium is more accurately determined by oxidimetric means, a value for it is not given. These recommended wave lengths compare well with those listed by Rodden (*19, 20*) but are more precisely determined. The same may be said with regard to the values given by Spedding *et al.* (*23*), except that these workers prefer the 740 and 795 mμ bands for neodymium and the 521,653, and 975 mμ bands for erbium. Although these neodymium bands are both

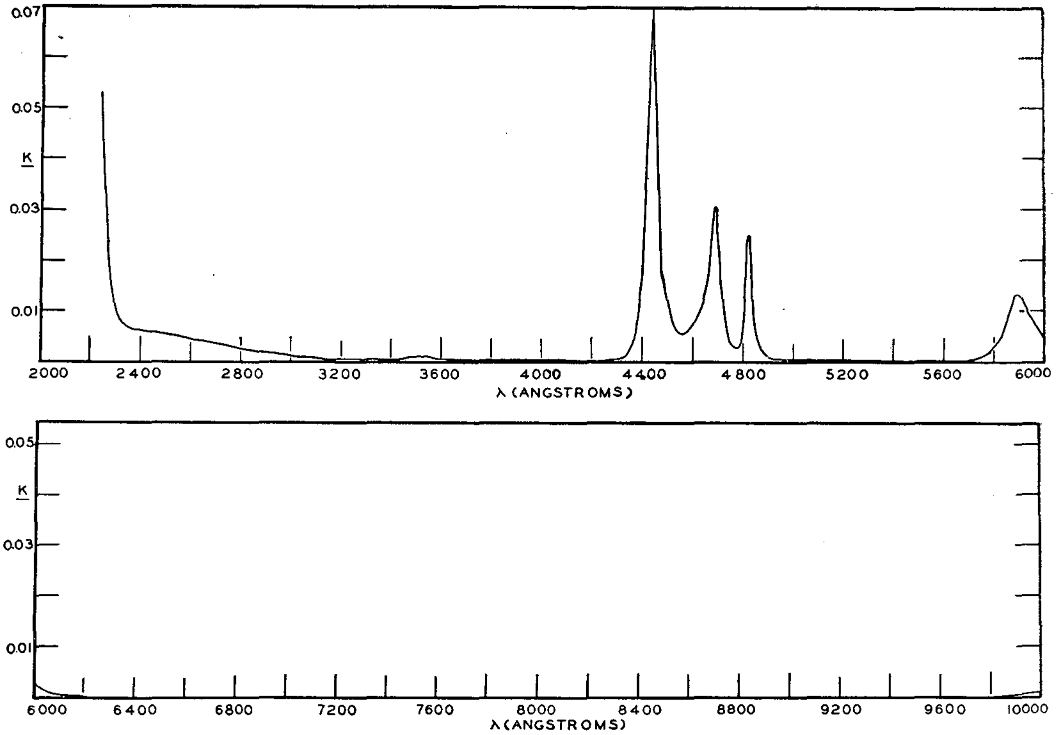

Figure 2. Absorption Spectrum of Praseodymium Chloride Solution

more intense than the recommended 5218 A. band, they are also more easily affected by excess anions. The recommended band for erbium is the most intense one in the useful spectrum. Data are included for acetates and nitrates in certain instances as well as for chlorides and perchlorates. The minimum sensitivity values given in Table II are the concentrations required to give a log I_0/I reading of 0.100.

A list of interferences is included in Table III. In two cases—interference of the 5230 A. erbium band with the 5218 A. neodymium band and of the 6800 A. neodymium band with the

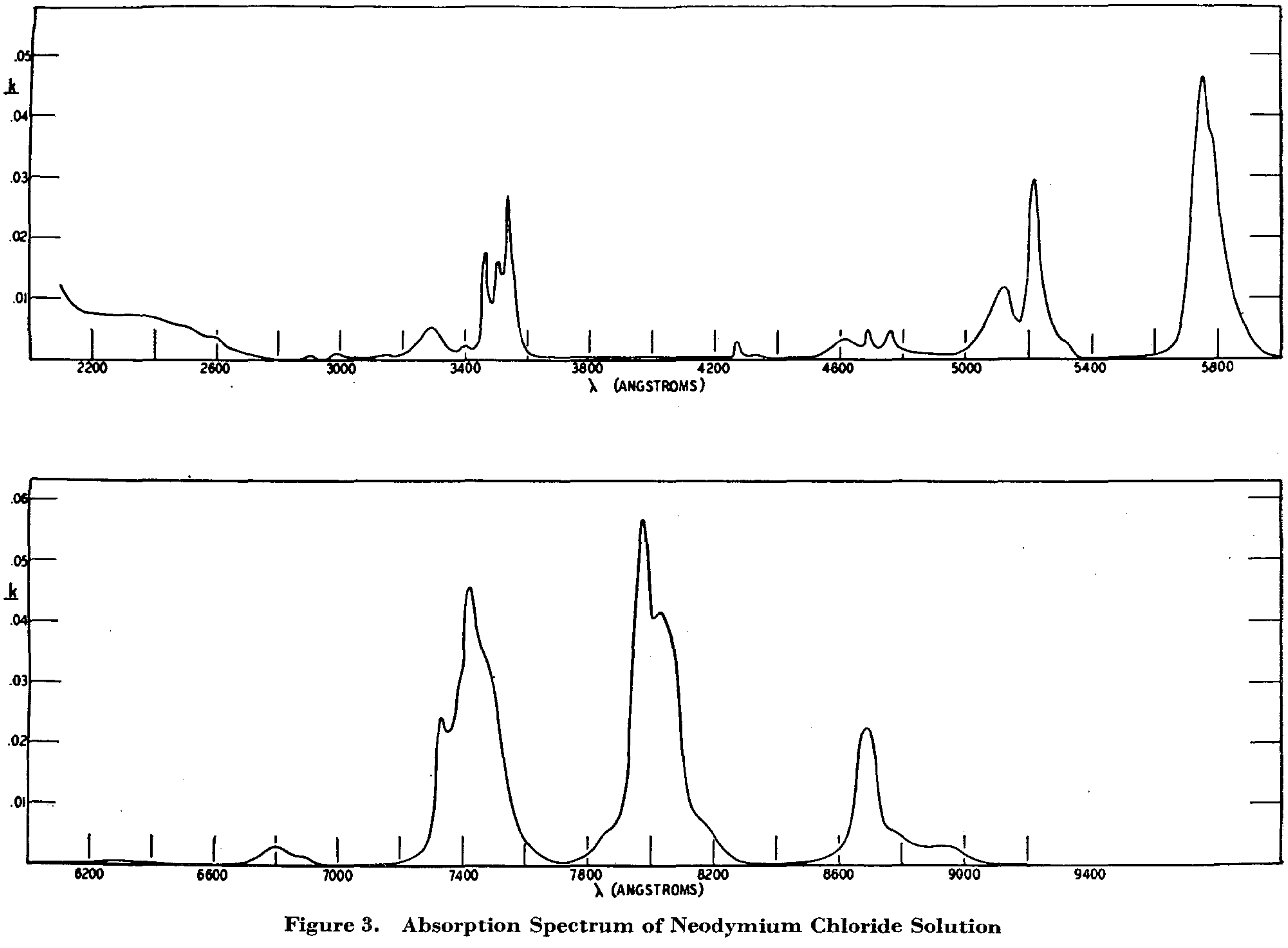

Figure 3. Absorption Spectrum of Neodymium Chloride Solution

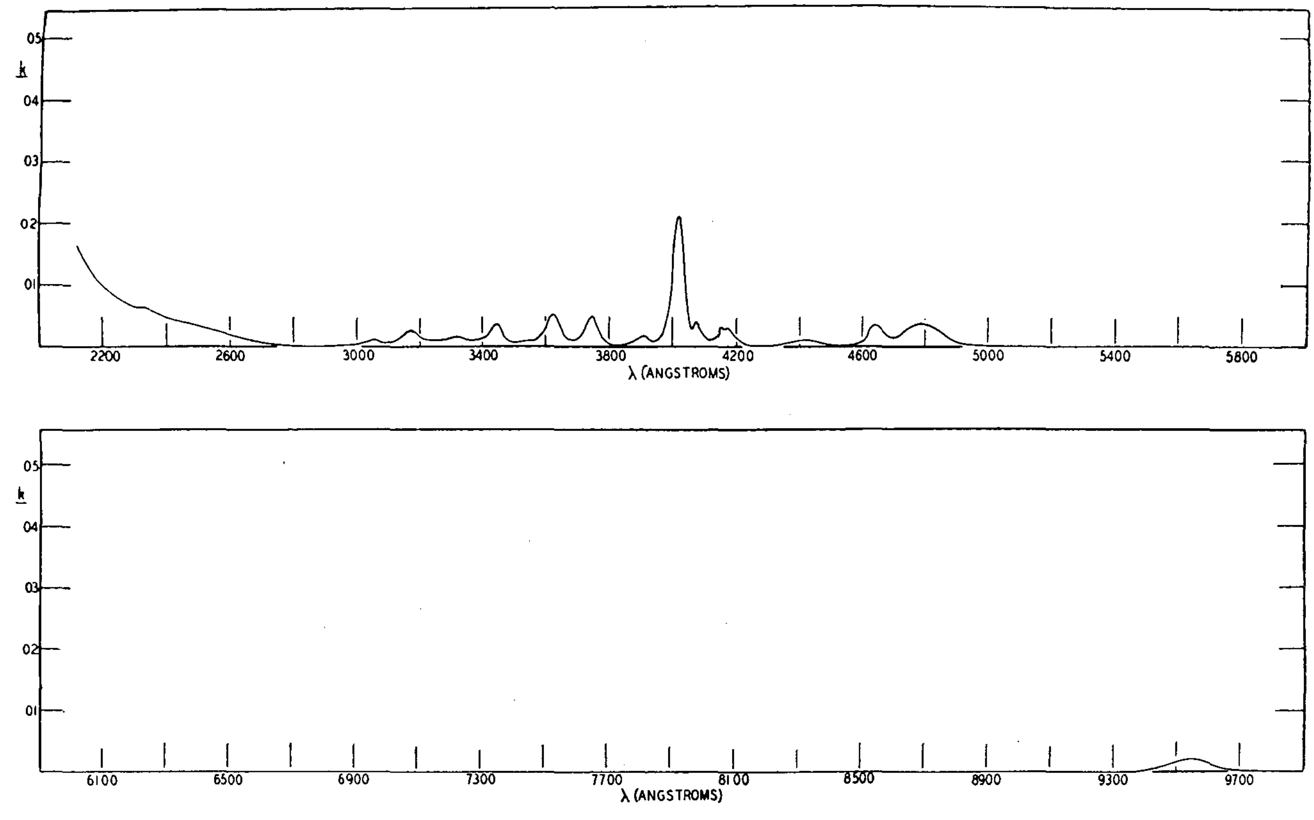

Figure 4. Absorption Spectrum of Samarium Chloride Solution

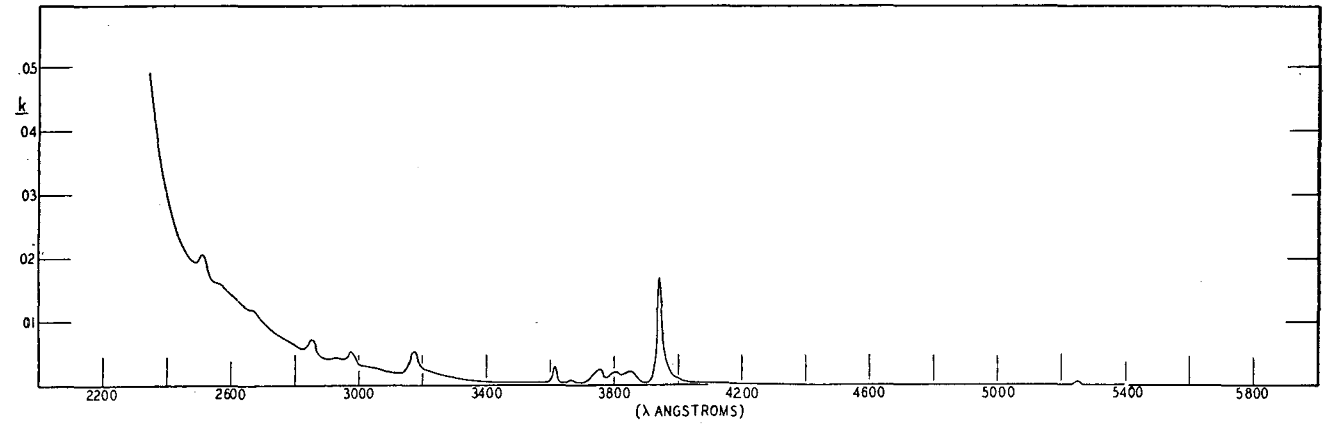

Figure 5. Absorption Spectrum of Europium Chloride Solution

6825 A. thulium band—such interference is unimportant because, except in natural minerals or in synthetically prepared samples, the simultaneous presence of neodymium and erbium or thulium is unlikely.

The correction formulas which may be applied for these interferences are discussed in a later section of this paper.

Table III. Interferences with Bands Chosen for Analytical Purposes

Salt Used	Wave Length, A.	k	Interfering Ion	k of Interfering Ion
$PrCl_3$	4445	0.0700	Sm^{+++}	0.0012
$NdCl_3$	5218	0.0300	Er^{+++}	0.00998
$SmCl_3$	4020	0.0210	Eu^{++}	0.0006
$EuCl_3$	3939	0.0192	Sm^{+++}	0.00101
$Tm(ClO_4)_3$	6825	0.0147	Nd^{+++}	0.00240
$Yb(ClO_4)_3$	9750	0.0111	Er^{+++}	0.00529

EFFECT OF SLIT WIDTH

For a given absorption band, the first measurements were always made with the sensitivity control at three turns from the extreme counterclockwise position. The slit width at some wave length was then recorded, so that readings could be duplicated if desired. The slit width was then increased by a factor of 2 by decreasing the sensitivity, and the band chosen for analytical purposes was measured again. A third measurement was made with the slit width decreased by a factor of 2 from the original value. The k values were calculated for all slit width values. Constancy in k was taken as indicative of complete resolution of the band. Although complete resolution of each band was desirable, it was not essential for a given material as long as the k value at a known slit width could be duplicated.

The slit width listed for each wave length in Table II is that at which complete resolution was obtained, or at which k was reproducible. The spectra of tripositive europium, erbium, and samarium are all affected by changes in slit width, and the bands are not completely resolved at any slit width obtainable with the Beckman instrument. For these materials, *k* values are reproducible at the slit widths listed in Table II. The spectra of the other ions, with the exception of gadolinium, in chloride and perchlorate solutions are unaffected by any obtainable slit width changes.

Gadolinium salt solutions presented unusual cases. At the ordinary operating slit width of the instrument (0.330 mm. at 2730 A.), such solutions showed only a broad, diffuse absorption band centering at 2730 A. A very slight decrease in slit width resolved this band into two bands. Accordingly investigations were extended using a technique which gives minimum slit width at a sacrifice in sensitivity. The sensitivity control was set at the counterclockwise limit, and the "check" switch was turned to 0.1 on the scale. The instrument was then balanced against the solvent by setting the density scale to zero and bringing the needle to zero through adjustment of the slit width control. The optical density was then read by introducing the sample into the light beam and balancing the instrument with the density scale control. As shown in Figure 6, the original band was thus split into six relatively sharp bands. The wave lengths characterizing these bands are in excellent agreement with those given by Prandtl and Scheiner (*17*). At 2730 A., the slit width obtained was 0.080 mm., corresponding to a nominal band width of 1.0 to 1.5 A.

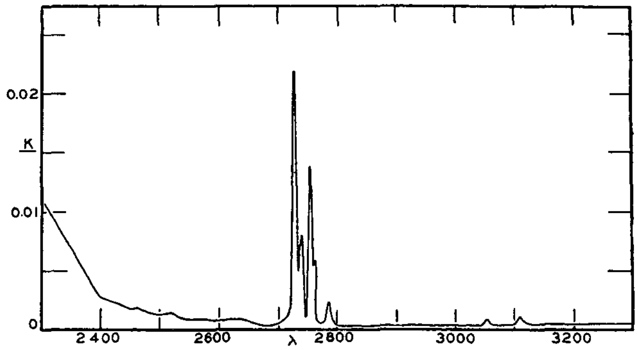

Figure 6. Absorption Spectrum of Gadolinium Chloride Solution

ADHERENCE TO BEER'S LAW

Rigorous adherence of the absorption bands of the rare earth metal ions to Beer's law has often been questioned (*18–21*, *25*). However, the systematic studies of Rodden (*19*, *20*) and the reports of others (*6*, *7*, *9–11*, *23*, *26*) indicate that deviations at lower concentrations at least are not marked for most materials. It was essential to this investigation to obtain further information on concentration effects.

A solution of the salt being studied was prepared in such concentration as to give a log I_0/I reading between 0.800 and 1.000. This solution was then diluted in a stepwise fashion, spectral readings being made at appropriate wave lengths at each dilution, until, as a lower limit, a solution was obtained for which I_0/I lay between 0.100 and 0.200. These upper and lower limits were chosen arbitrarily because of the enhanced accuracy of the instrument in this range. In no cases were concentrations in excess of 72 grams of metal per liter used, and in the majority of the cases the upper limit was 25 grams. In this fashion, chloride solutions and erbium perchlorate solutions were investigated systematically. Plots of log I_0/I against concentration were made to establish the independence of absorption coefficient, *k*, and concentration. Beer's law requires the existence of a linear relation between log I_0/I and concentration. Data summarized in Table IV indicate clearly that over the concentration ranges investigated Beer's law is obeyed within 1% at the absorption peaks of analytical importance for all the materials studied except gadolinium. With gadolinium, deviations up to ±10% were noted. This is undoubtedly due to the difficulty of resolving the absorption in the vicinity of 2730 A. and the consequent inaccuracy in spectrophotometric readings. However, it is apparent that, with this exception, estimation of the rare earths by means of absorption spectra data is feasible.

EFFECTS OF OTHER IONS

Because of the difficulty of removing excess acid through evaporation, it was of interest to determine whether accurate

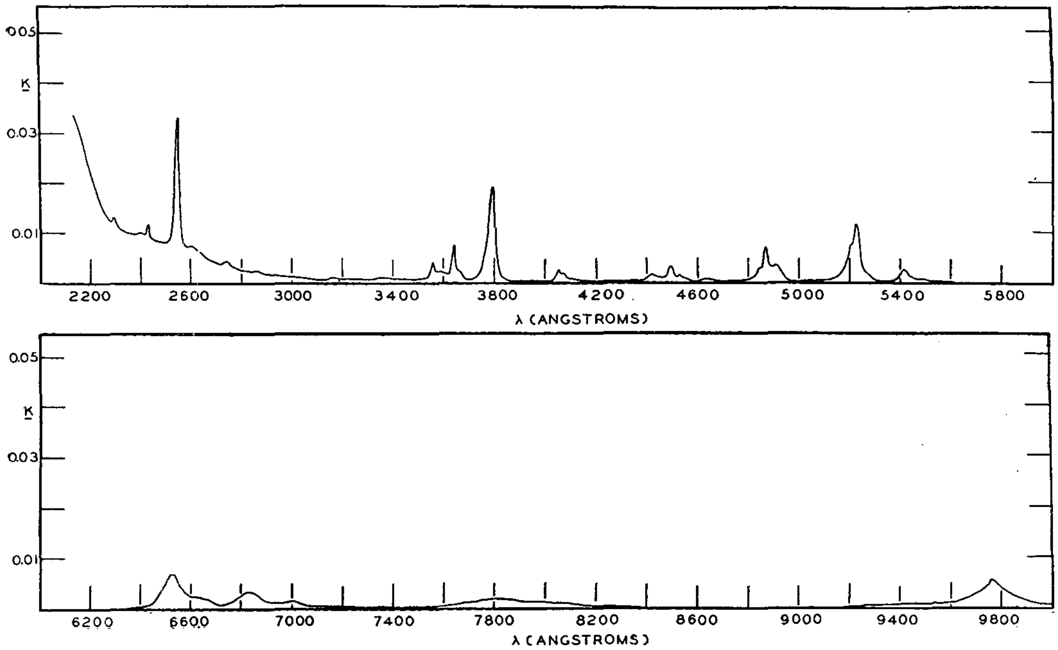

Figure 7. Absorption Spectrum of Erbium Perchlorate Solution

Table IV. Beer's Law Determinations

Rare Earth Salt	Wave Length A.	Slit Width Mm.	Concentration G. metal/liter	log I_0/I
$PrCl_3$	4445	0.025	12.5	0.819
			6.25	0.410
			2.50	0.164
			1.25	0.082
$NdCl_3$	5218	0.013	25.0	0.741
			12.5	0.370
			5.0	0.148
$SmCl_3$	4020	0.045	25.0	0.534
			12.5	0.268
			5.0	0.106
$EuCl_3$	3939	0.054	25.0	0.450
			12.5	0.224
$GdCl_3$	2730	0.250	24.99	0.360
			12.495	0.171
$Er(ClO_4)_3$	3792	0.030	18.48	0.881
			9.24	0.441
			4.62	0.222
		0.060	18.48	0.800
			9.24	0.410
			4.62	0.207
$TmCl_3$	6825	0.018	53.91	0.725
			26.96	0.363
			13.48	0.182
$YbCl_3$	9750	0.026	72.43	0.800
			36.21	0.401
			18.11	0.200

results could be obtained in the presence of unremoved acid or excess of the characteristic anion. Acid-free chloride solutions containing the equivalent of 25 grams of the metal per liter were prepared by the technique previously outlined. Aliquots were then treated with varying quantities of standard hydrochloric acid or ammonium chloride solutions, the total rare earth content in a given series being kept constant by appropriate dilution. For each solution, the absorption coefficient was then measured at the predetermined analytical wave length, the value for the pure chloride solution being used for comparison.

For the cerium earths, addition of chloride ion had no measurable effect. This is shown by the data for neodymium as summarized in Table V. Quantitative removal of excess hydrochloric acid is thus not essential to spectrophotometric estimation of these elements. On the other hand, yttrium earth chloride solutions—e.g., those of erbium, thulium, ytterbium—were sensitive to the presence of excess chloride ion. Absorption coefficients varied with both chloride content and time. Removal of excess hydrochloric acid permitted reproducible readings with both thulium and ytterbium, if measurements were made within 2-day intervals.

Table V. Effects of Chloride Ion on Absorption of Neodymium Chloride Solutions at 5218 A.

Material Added	Mole Ratio Cl^-: Nd^{+++}	Absorption Coefficient, k
HCl	0.00	0.0300
	0.0397	0.0300
	0.0993	0.0298
	0.993	0.0300
	9.939	0.0300
NH_4Cl	0.000	0.0300
	2.00	0.0300
	4.00	0.0302
	10.00	0.0306

Erbium chloride solutions gave varying absorption coefficients even when free of excess chloride ion. Perchlorate solutions, however, gave reproducible readings at 3792 A., either when pure or when the mole ratio of added excess perchloric acid to erbium was as much as 4 to 1. For any mixture containing erbium, a perchlorate medium is recommended. Addition of excess perchloric acid was also without effect in other cases.

The effects of individual rare earth ions upon the absorption spectra of each other have never been evaluated systematically, probably because the rarities of these materials have precluded their use in synthetic mixtures in any but exceptional cases. In this investigation, a few such synthetic mixtures were studied. In view of the excellent results obtained during the spectrophotometric analysis of these mixtures, it appears that the individual materials exerted little or no effect upon each other, with the exception of an increase in the absorption value for erbium when ytterbium or thulium ion was present. This does not rule out the possibility of interactions at higher concentrations, as reported by Quill, Selwood, and Hopkins (*18*).

SPECTROPHOTOMETRIC ESTIMATION OF RARE EARTH METAL IONS

Based upon the above observations, the following procedure is recommended for the spectrophotometric estimation of rare earth metal ions that show characteristic absorption bands.

The freshly ignited, weighed (1 to 3 grams) oxide sample is slurried with water and dissolved in a slight excess of hydrochloric (or perchloric) acid. Excess acid is removed by evaporating to dryness, taking up the residue in water, and re-evaporating. The final residue is taken up in water and the resulting solution is diluted to a suitable volume (say 100 ml.). The log I_0/I values are then determined at the wave lengths and slit widths summarized in Table II if a mixture is being studied or at an individual recommended wave length if but a single element is to be estimated. (These values apply rigidly only to the instrument used by the authors. Anyone using another instrument should obtain his own set of calibration data.) Although in the authors' experience duplication of these wave lengths has been achieved in 95% of the cases studied, the sharp natures of the bands may make peak settings difficult with some instruments. It is recommended that no less than five readings be made in the vicinity of each peak at slightly differing wave-length settings and that the maximum log I_0/I value so obtained be used in subsequent calculations.

The quantity of rare earth ion present is then calculated from the measured log I_0/I value and the characteristic k value as summarized in Table II, using the expression

$$\text{Grams of R}^{+++}\text{ per liter} = \frac{\log I_0/I}{k}$$

use of 1-cm. cells being assumed.

In cases where other ions interfere (Table III), necessary corrections may be made by calculating log I_0/I due to the interfering ion and subtracting this value from the measured log I_0/I. This correction factor is obtained by multiplying the concentration of the interfering ion by the absorption coefficient of this ion at the wave length in question. Thus, to determine the amount of a rare earth, A, present when another one, B, interferes, one uses the expression

$$\text{Grams of } A \text{ per liter} = \frac{\log I_0/I - (\text{grams of } B \text{ per liter} \times k_B)}{k_A}$$

Values of k listed in Table III are for such calculations.

The mutual interference between the 3939 A. band for europium and the 4020 A. band for samarium, the only strong bands available for these ions, presents a more difficult problem. Two alternatives are suggested. The first involves chemical estimation of europium by oxidimetric means and corresponding correction of the samarium determination. The second, which is simpler and of equal accuracy, involves a preliminary calculation of the quantity of material present in the larger amount. This quantity is then used to correct the measured concentration of the other ion in the fashion outlined above. The resulting concentration of the second ion is then used in arriving at a more accurate value for the first. In three such calculations, accuracies within 1% are obtained.

A sample calculation follows for a mixture of europium chloride and samarium chloride (see Table VI, Item 3).

1. Grams of Sm/liter $= \dfrac{0.311}{0.0210} = 14.80$

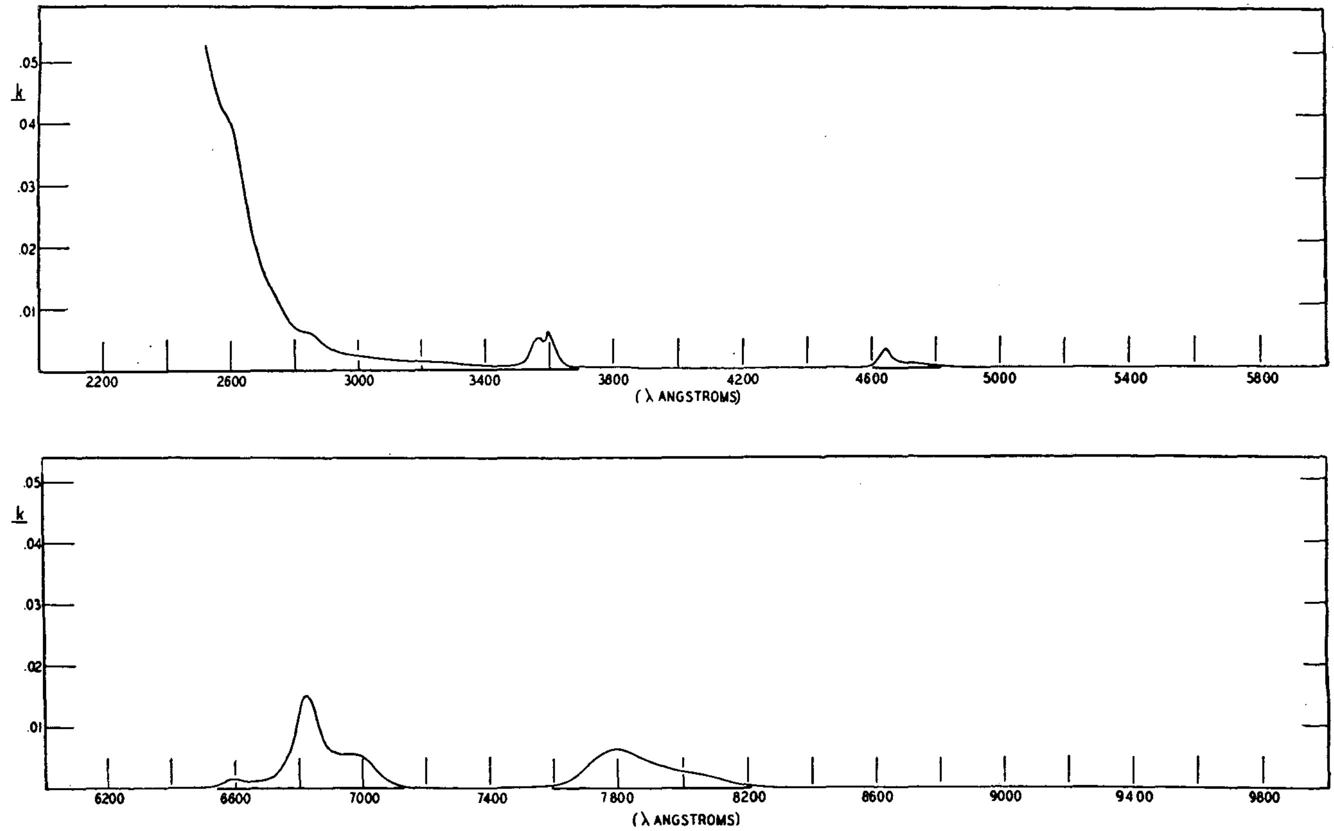

Figure 8. Absorption Spectrum of Thulium Chloride Solution

2. $$\text{Grams of Eu/liter} = \frac{0.261 - (14.80 \times 0.001)}{0.0192} = 12.84$$

3. $$\text{Grams of Sm/liter} = \frac{0.311 - (12.82 \times 0.0006)}{0.0210} = 14.48$$

Table VI. Representative Analyses of Rare Earth Materials

No.	Materials Present	Amount Present	Amount Found
		G. metal/liter	*G. metal/liter*
1[a]	Pr	1.62	1.63
	Nd	4.84	4.86
	Sm	9.10	9.19
2[a]	La	5.65	...
	Nd	7.20	7.15
	Sm	6.80	6.85
3[a]	Sm	14.54	14.48
	Eu	12.95	12.84
4[a]	Er	12.3	12.4
	Yb	26.3	26.2
5[a]	Er	6.15	6.65
	Yb	13.2	13.1
6[a]	Er	2.46	2.89
	Yb	47.4	47.5
7[b]	Er	3.02	4.80
	Tm	9.40	9.38
	Yb	5.95	5.94
8[c]	Nd	5.02	5.05
9[c]	Sm	8.69	8.70

[a] Synthetic mixture.
[b] Analyzed spectrophotometrically by C. J. Rodden.
[c] Analyzed gravimetrically by oxalate precipitation.

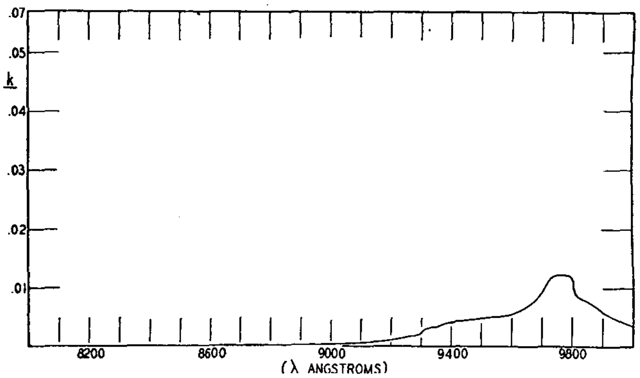

Figure 9. Absorption Spectrum of Ytterbium Chloride Solution

Data summarized in Table VI indicate the applicability of the general method. Mixtures analyzed were either synthetic mixtures prepared from known quantities of pure materials or samples for which independent analyses were available as indicated.

It is therefore apparent that an accuracy of ±1% may be expected for materials discussed in this paper, except gadolinium and erbium. Precision is excellent. The procedure is adaptable to the analysis of a variety of samples. It is particularly applicable to analyses in the presence of scandium, yttrium, lanthanum, lutecium, and thorium, because these ions show no absorption in the range 2200 to 10,000 A. Estimation of these elements must be effected by other means, and cerium is better determined by oxidimetry. For routine work in following fractionations or tracing the behavior of an element in a series of reactions, the procedure is invaluable because of its rapidity and accuracy. It must be emphasized, however, that unlike many colorimetric procedures, this one is not adaptable to a micro scale. The characteristic absorption exhibited by the rare earth metal ions is not sufficiently intense to permit such an extension.

ACKNOWLEDGMENT

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Spectrographic Analysis of Coal and Coal Ash

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Coal can be analyzed on the spectrograph for per cent ash and composition of ash in a matter of a few minutes, using the total energy method. The composition of the ash so determined can be used to calculate ash softening temperatures. This analysis can be made in sufficiently short a time to control tipple and washing operations for preparation of coal to meet specifications. This spectrographic method can be readily adapted to the analysis of rocks, minerals, and inorganic chemicals of all kinds.

SPECTROGRAPHIC methods are being developed for quantitatively analyzing all types of rocks and minerals (*1–3*). The rapidity with which the analyses can be made, the positive identification of the element sought, and, in numerous instances, the accuracy of the data obtained make this method of analysis desirable.

The total energy method (*2*), in which the sample is burned completely in a direct current arc, was used in this investigation for developing a method for the analysis of ash in coal. Internal standards are not needed in this method. The wide range of volatility of the elements in coal ash gives the total energy method an advantage over the internal standard method for this type of sample. The internal standard method requires many more standards than does the total energy method; a very few standards suffice for the analysis of several elements in a wide range of sample types, using the total energy method.

APPARATUS AND METHODS

The instruments used are the large Littrow prism-type spectrograph, conventional direct current excitation source unit, and a projection comparator densitometer.

In the analysis of raw coal the samples are ground to 200-mesh and after being thoroughly mixed are weighed directly into a high-purity graphite spectroscopic electrode. Five milligrams of sample are employed. These electrodes are then placed in a small electric muffle for about 10 minutes at 550° C. to drive off the volatile matter. The spectra of four samples and one standard sample are then photographed on the same spectroscopic plate. The electrode holding the sample forms the positive pole of a 220-volt direct current arc drawing 12 to 15 amperes. All spectra are modulated at the slit by a logarithmic step sector, revolving at 2500 r.p.m. Samples are burned to completion.

From this plate the densities of the various spectral lines are read on a densitometer and the following oxides, the major constituents of the ash, are quantitatively determined: silicon, ferric, aluminum, titanium, calcium, magnesium, sodium, and potassium. The per cent ash will therefore be the sum total of the above determinations plus the sulfur trioxide, which is discussed below.

The standard employed on these tests was a sample of West Virginia coal, C-5. The coal and ash were analyzed by conventional methods in the authors' laboratory. The analysis of this ash sample was also determined on the spectrograph, employing a synthetic standard made from oxides and carbonates of known purity (Table I). The chemical analysis of C-5 ash was used as a basis for calculating the spectrographic analysis of the unknown samples.

After 70 coal samples had been analyzed, the data were tabulated and the difference between the burning method and spectrographic method was determined. On plotting the per cent ash in coal against the plus or minus difference, it was found that the spectrographic method invariably gave high values on low-ash coals and low values on coals whose ash content was considerably greater than the coal used as a standard.

The variation for each element was directly proportional to the ratio of concentration of that element in the standard, versus

Table I. Comparison of Chemical and Spectrographic Analysis of C-5 Coal and C-5 Coal Ash

	SiO_2 %	Fe_2O_3 %	Al_2O_3 %	TiO_2 %	CaO %	MgO %	Na_2O %	K_2O %	Li_2O %	Total Alkalies %
					Chemical Analysis of Ash					
	57.37	6.63	27.16	1.03	1.00	0.92	1.96	1.39	With Na_2O	3.35
					Spectrographic Analysis of Ash					
	55.90	6.11	28.86	1.39	0.95	0.96	1.16	2.11	0.47	3.74
	55.99	5.92	28.93	1.43	0.91	0.99	1.14	1.90	0.48	3.52
	58.23	6.49	26.40	1.42	0.83	0.85	1.12	2.00	0.44	3.56
	58.21	6.18	26.41	1.46	0.95	0.87	1.12	1.80	0.45	3.37
							1.16			
							1.16			
Av.	57.08	6.17	27.65	1.42	0.91	0.91	1.14	1.95	0.46	
					Analysis of Coal Calculated from Spectrographic Analysis of Ash					
	8.42	0.91	4.07	0.21	0.13	0.13	0.16	0.28	0.067	
					Spectrographic Analysis of Coal					
	8.01	0.87	3.94	0.24	0.12	0.13	0.16	0.30	0.069	
	7.84	0.84	3.89	0.21	0.12	0.12	0.16	0.26	0.060	
	8.39	0.97	4.23	0.25	0.13	0.13	0.17	0.32	0.082	
	8.21	0.94	4.18	0.22	0.13	0.12	0.17	0.27	0.082	
	8.63	1.05	4.38	0.26	0.14	0.14	0.16	0.33	0.072	
	8.45	1.02	4.32	0.23	0.14	0.13	0.16	0.29	0.072	
Av.	8.25	0.94	4.15	0.23	0.13	0.13	0.16	0.29	0.072	

the concentration of the element in the sample being analyzed. Of the three major elements, iron oxide showed this effect the least; silicon dioxide showed the greatest difference at low concentrations and considerable difference at higher concentrations. The aluminum oxide showed the greatest difference at high concentrations. These differences are too great to be tolerated and need to be corrected for in some manner.

Several of the coals were again analyzed and the weight of the sample taken was adjusted so that the weights of the silicon dioxide and aluminum oxide in the standard and the sample were approximately the same. This procedure gave much better checks between the burning method for total ash and the spectrographic method.

From preliminary data it appears possible to set up a concentration correction curve for each of the elements. This curve can be obtained by spectrographically analyzing dilutions of the standard with high-purity carbon and by analyzing concentrations of the elements sought by adding high-purity oxides to the standard ash. The difference between the calculated analysis and the spectrographic analysis is plotted against the concentration of each element. The equal weights of the sample and standard—for example, 5 mg.—may then be taken and the proper correction for difference in concentration taken from the curves and applied to the analytical data so obtained.

ANALYTICAL DATA

Some 70 channel samples representative of the coal throughout West Virginia have been analyzed by this method. The per cent ash determined spectrographically checks the value obtained by the usual method of burning 1 gram of coal at 740° C. within the check limits of that method. At the same time the spectrochemical method gives a complete ash analysis in a fraction of the time required for a chemical analysis. It is sufficiently accurate for most purposes.

Table II presents the results of the analysis of coal by the spectrographic method, the per cent ash determined by burning 1 gram at 740° C., and an occasional chemical analysis for comparative purposes. Ash samples C-3, 10, 11, 13, 16, 19, 22, 25, 49, and 53 were analyzed by the usual chemical methods and this type of analysis is designated as "wet" in Table II. No attempt was made to make a comparative study of the spectrographic and chemical analysis of these ten coals, because the spectrographic analysis was made on coal while the chemical analysis was made on ash. Even so, numerous instances of good agreement between the two methods are shown in Table II. On the first runs, samples C-1 to C-53, the volatile matter was not driven off prior to arcing. It has since been determined that in the rapid evolution of this material in some coals there was transportation of some of the sample out of the electrodes without excitation at the time of ignition of the arc. Coke remaining in partially ignited coal also transports unexcited particles through the arc. This cannot be tolerated using the total energy method of quantitative spectrochemical analysis. Samples C-66 to C-98 were preheated to 550° for 10 minutes.

A cumulative distribution curve of the algebraic difference between the per cent ash determined by the burning method and that by the spectrographic method was prepared on probability paper (Figure 1). The difference in the per cent ash by the two methods shows a normal distribution. The two methods will give results that check with ±1% in 97% of the number of tests.

A primary objective of this investigation was to develop a rapid method for the analysis of coal ash. The composition of the ash can be determined spectrographically on either the raw coal or the ash. Coals C-66 to C-98 were analyzed by both methods. The coals were ashed at 740° C. to obtain the ash samples. Generally speaking, the values obtained by the two methods agree (Table III), although the aluminum oxide as determined on coal is invariably higher than the value obtained on ash. The reason for this is not apparent at the moment, but some of the aluminum in the coal may escape on burning; and the coal may affect the relative intensity of the aluminum lines.

FACTORS AFFECTING ACCURACY OF SPECTROCHEMICAL METHODS

Any analytical method employing a standard will give results of no greater accuracy than the analytical data of the standard.

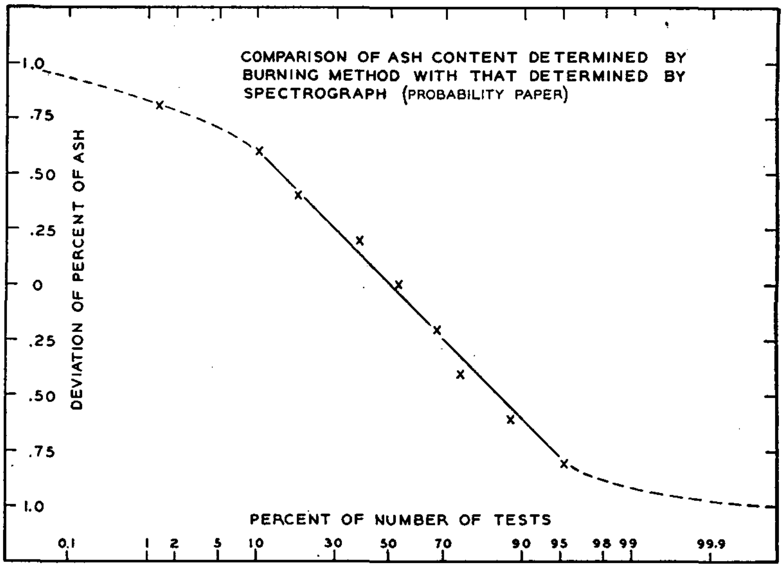

Figure 1

After the completion of this work it was found advisable to make synthetic standards from chemicals of known spectrographic purity. Table I lists several spectrographic analyses of C-5 ash using a synthetic mixture as a standard, and also a chemical analysis of C-5 ash. The spectrographic value for ferric oxide is 0.4% less than the chemical analysis, whereas the spectrographic value for titanium oxide is 0.4% more than the chemical analysis. Apparently 0.4% of the sample reported as ferric oxide in the chemical analysis was really titanium oxide. The chemical separation of sodium and potassium oxides was not complete. Similar differences in these oxides, especially sodium oxide, were noted when analyzed samples from nationally known laboratories were checked spectrographically. Very few samples are available with sufficiently accurate analysis for spectrochemical standards, especially in the case of elements that are present in concentrations of less than 0.2%. Twenty or more elements occur in coal ash with concentrations approaching 0.1%, and for maximum accuracy it is necessary to take these elements into consideration. There is a definite need for several accurate analyses of coal ash. Synthetic mixtures of chemicals of known spectrographic

Table II. Spectrographic Analysis of Coals

Sample No.	Type of Analysis[a]	SiO_2, %	Fe_2O_3, %	Al_2O_3, %	TiO_2, %	CaO, %	MgO, %	MnO, %	Na_2O, %	K_2O, %	Total Ash Spec.	Total Ash Burning	% Difference +	% Difference −
C-1	Spec.	3.65	1.58	1.97	0.06	0.20	0.07	0.0013	0.37	0.17	8.07	8.14		0.07
C-2	Spec.	3.94	1.67	1.97	0.07	0.20	0.10	0.0014	0.23	0.14	8.32	8.19	0.13	
C-3	Spec.	3.02	0.85	1.50	0.04	0.30	0.11	...	0.12	0.15	6.09	5.89	0.20	
C-3	Wet	2.24	1.10	0.89	0.07	0.66	0.17	...	0.06	0.10	...	...		
C-8	Spec.	1.17	0.70	1.21	0.01	0.15	0.04	0.0010	0.36	0.20	3.84	3.47	0.37	
C-9	Spec.	2.82	0.78	1.67	0.06	0.40	0.08	0.0026	0.57	0.31	6.69	6.42	0.27	
C-10	Spec.	4.23	2.08	2.10	0.06	0.15	0.06	0.0014	0.50	0.20	9.38	9.09	0.29	
C-10	Wet	4.06	1.98	2.04	0.18	0.22	0.08	None	0.04	0.06	...	...		
C-11	Spec.	5.05	6.46	1.93	0.10	0.22	0.05	0.0019	0.21	0.15	14.17	15.11		0.94
C-11	Wet	5.18	6.23	2.41	0.30	0.17	0.03	0.004	0.32	0.23	...	...		
C-12	Spec.	6.40	1.35	3.16	0.16	0.16	0.07	0.0013	0.61	0.17	12.08	11.71	0.37	
C-13	Spec.	3.20	1.59	1.86	0.07	0.15	0.06	0.0010	0.61	0.18	7.73	8.14		0.41
C-13	Wet	3.27	2.60	1.59	0.07	0.15	0.05	None	0.06	0.05	...	...		
C-14	Spec.	7.07	1.59	3.45	0.15	0.15	0.07	0.0010	0.61	0.15	13.24	12.86	0.38	
C-15	Spec.	4.42	5.35	1.93	0.07	0.19	0.07	0.0013	0.38	0.30	12.70	12.30	0.40	
C-16	Spec.	4.12	2.14	2.00	0.06	0.14	0.07	0.0010	0.38	0.11	9.02	9.41		0.39
C-16	Wet	4.00	2.57	1.94	0.10	0.20	0.08	Trace	0.27	0.05	...	...		
C-17	Spec.	6.30	1.34	3.01	0.13	0.14	0.07	0.0009	0.49	0.18	11.67	11.86		0.19
C-18	Spec.	4.52	5.00	2.06	0.08	0.15	0.07	0.0012	0.25	0.14	12.27	13.03		0.76
C-19	Spec.	2.95	1.92	1.74	0.06	0.16	0.05	0.0009	0.38	0.11	7.37	7.30	0.07	
C-19	Wet	2.98	2.12	1.74	0.10	0.14	0.04	Trace	0.03	0.04	...	...		
C-20	Spec.	3.57	0.85	1.91	0.07	0.18	0.05	0.0010	0.34	None	6.97	7.09		0.12
C-21	Spec.	5.28	5.69	2.03	0.10	0.17	0.07	0.0013	0.18	0.10	13.62	14.25		0.63
C-22	Spec.	2.84	1.92	1.91	0.05	0.16	0.05	0.0010	0.29	0.09	7.31	7.86		0.55
C-22	Wet	3.02	2.15	1.72	0.05	0.19	0.05	None	0.41	0.04	...			
C-23	Spec.	7.05	1.74	3.63	0.17	0.20	0.07	0.0012	0.37	0.13	13.36	12.79	0.57	
C-24	Spec.	4.16	3.30	1.94	0.06	0.15	0.05	0.0010	0.18	0.09	9.93	10.54		0.61
C-25	Spec.	3.50	1.74	1.97	0.07	0.15	0.06	0.0010	0.38	0.12	7.99	8.43		0.44
C-25	Wet	3.65	2.34	1.81	0.06	0.19	0.05	Trace	0.06	0.02	...			
C-26	Spec.	1.48	0.28	1.16	0.02	0.11	0.05	0.0010	0.48	0.11	3.69	3.01	0.68	
C-30	Spec.	0.80	0.84	0.53	0.01	0.17	0.06	0.0010	0.22	None	2.63	2.05	0.58	
C-31	Spec.	0.68	0.84	0.20	None	0.08	0.08	0.0026	0.50	None	2.38	2.19	0.19	
C-36	Spec.	2.75	1.76	1.56	0.05	0.16	0.07	0.0014	0.63	0.23	8.39	7.71	0.68	
C-39	Spec.	21.15	2.66	7.02	0.25	0.14	0.27	0.0017	0.56	0.54	32.59	31.86	0.73	
C-40	Spec.	4.55	0.38	3.04	0.09	0.17	0.07	0.0006	0.19	0.10	8.59	8.41	0.18	
C-41a	Spec.	4.64	2.71	2.66	0.09	0.30	0.07	0.0022	0.35	0.18	10.93	11.40		0.47
C-41b	Spec.	6.34	2.58	3.13	0.11	0.22	0.08	0.0013	0.38	0.19	13.03	12.88	0.15	
C-48	Spec.	4.54	1.97	2.63	0.09	0.19	0.06	0.0018	0.18	0.32	9.98	10.38		0.40
C-49	Spec.	2.05	2.19	1.23	0.04	0.21	0.06	0.0017	0.15	None	5.93	6.11		0.18
C-49	Wet	1.77	2.74	0.84	0.09	0.23	0.02	0.0014	0.14	0.03	...	...		
C-50	Spec.	5.12	9.02	4.37	0.07	0.20	0.06	0.0018	0.27	0.15	19.22	19.10	0.12	
C-52	Spec.	5.14	2.94	3.15	0.09	0.16	0.07	0.0015	0.23	0.18	11.96	11.12	0.84	
C-53	Spec.	1.76	2.15	0.95	0.03	0.11	0.06	0.0014	0.22	0.12	5.40	5.03	0.37	
C-53	Wet	1.46	2.24	0.64	0.10	0.21	0.02	0.0032	0.10	0.02	...	...		
C-66	Spec.	14.38	1.10	6.91	0.23	0.20	0.19	0.0025	0.31	0.22	23.5	23.9		0.4
C-67	Spec.	15.40	4.18	9.17	0.21	0.24	0.42	0.0031	0.41	0.31	30.34	31.20		0.86
C-68	Spec.	7.37	1.20	3.71	0.12	0.24	0.10	0.0017	0.29	0.17	13.1	13.6		0.5
C-69	Spec.	6.39	0.58	3.32	0.09	0.28	0.10	0.0014	0.17	0.09	10.9	10.1	0.8	
C-70	Spec.	6.90	1.19	2.84	0.10	0.25	0.08	0.0014	0.20	0.12	11.70	12.49		0.79
C-71	Spec.	2.72	0.25	2.10	0.07	0.20	0.04	0.0005	0.22	0.11	5.60	5.07	0.53	
C-72	Spec.	5.09	0.45	4.00	0.10	0.72	0.13	0.0015	0.30	0.14	10.9	11.5		0.6
C-73	Spec.	5.57	0.68	3.05	0.09	0.10	0.08	0.0008	0.23	0.14	9.8	9.6	0.2	
C-74	Spec.	5.15	0.70	2.90	0.11	0.08	0.08	0.0006	0.29	0.17	9.4	10.1	0.7	
C-75	Spec.	7.16	0.79	5.12	0.12	0.10	0.14	0.0005	0.40	0.25	14.1	14.2		0.1
C-76	Spec.	10.31	1.08	6.13	0.16	0.08	0.18	0.0009	0.43	0.29	18.6	18.2	0.4	
C-77	Spec.	7.83	0.73	5.12	0.13	0.08	0.12	0.0006	0.41	0.26	14.7	15.2		0.5
C-78	Spec.	7.55	1.11	4.00	0.13	0.06	0.11	0.0006	0.42	0.30	13.7	14.4		0.7
C-80	Spec.	6.46	0.40	2.96	0.11	0.07	0.05	0.0005	0.23	0.11	10.3	10.2	0.1	
C-81	Spec.	5.13	0.41	2.83	0.12	0.07	0.05	0.0006	0.23	0.10	8.90	8.60	0.30	
C-82	Spec.	5.21	0.66	2.64	0.07	0.10	0.08	0.0006	0.21	0.16	9.1	9.4		0.3
C-83	Spec.	5.58	0.31	2.80	0.10	0.15	0.08	0.0013	0.24	0.15	9.4	9.0	0.4	
C-84	Spec.	5.79	0.22	2.64	0.13	0.07	0.05	0.0005	0.20	0.14	9.2	8.8	0.4	
C-85	Spec.	2.11	0.23	0.84	0.05	0.07	0.02	0.0005	0.19	0.12	3.6	4.1		0.5
C-86	Spec.	2.82	0.84	2.27	0.05	0.06	0.06	0.0005	0.32	0.21	6.6	6.8		0.2
C-88	Spec.	1.31	0.21	0.86	0.03	0.08	0.01	None	0.24	0.12	3.8	3.8	0.0	0.0
C-89	Spec.	1.49	0.35	0.93	0.04	0.07	0.02	0.0004	0.24	0.11	3.2	4.1		0.9
C-90	Spec.	1.48	0.97	1.40	0.05	0.08	0.02	0.0009	0.27	0.11	4.4	4.4	0.0	0.0
C-91	Spec.	4.40	1.38	1.96	0.07	0.14	0.07	0.0018	0.29	0.13	8.44	7.70	0.74	
C-92	Spec.	1.28	0.97	1.19	0.03	0.06	0.02	0.0005	0.31	0.11	3.9	3.5	0.4	
C-93	Spec.	2.11	0.66	1.96	0.07	0.38	0.06	0.0010	0.41	0.11	5.7	6.3		0.6
C-94	Spec.	2.56	0.74	2.07	0.09	0.62	0.07	0.0013	0.29	0.11	6.5	6.5	0.0	0.0
C-95	Spec.	2.22	0.80	2.07	0.08	0.31	0.05	0.0010	0.22	0.09	5.8	5.6	0.2	
C-96	Spec.	1.08	0.32	1.27	0.04	0.08	0.02	None	0.25	None	3.0	2.4	0.6	
C-97	Spec.	0.94	0.27	1.16	0.04	0.07	0.02	None	0.24	None	2.7	2.4	0.3	
C-98	Spec.	1.15	0.20	1.05	0.03	0.07	0.01	None	0.50	0.13	3.1	2.5	0.6	

[a] Spec., spectrochemical; wet, chemical.

Table III. Comparison of Spectrographic Analysis of Coal and Ash

(Analysis of coal calculated to pure ash or combustible matter–free basis)

Sample No.	SiO_2		Fe_2O_3		Al_2O_3		TiO_2		CaO		MgO		MnO		Na_2O,	K_2O,	CuO,	NiO,	V_2O_5,	Cr_2O_3,
	Ash	Coal	Ash	Coal	Ash	Coal	Ash	Coal	Ash	Coal	Ash	Coal	Ash	Coal	Coal	Coal	Ash	Ash	Ash	Ash
C-66	63.37	61.19	6.04	4.68	24.64	29.40	0.91	0.98	1.00	0.85	0.81	0.81	0.010	0.010	1.31	0.94	0.013	0.010	0.045	0.026
C-67	49.54	50.82	12.84	13.79	27.16	30.26	0.86	0.69	0.70	0.62	1.15	0.79	0.010	0.010	1.35	1.02	0.018	0.010	0.043	0.029
C-68	54.50	56.26	7.90	9.16	27.16	28.32	0.86	0.92	1.60	1.83	0.76	0.76	0.011	0.012	2.21	1.30	0.014	0.009	0.040	0.026
C-69	54.50	58.62	3.80	5.32	24.64	30.46	0.86	0.83	2.40	2.57	0.73	0.92	0.011	0.013	1.56	0.83	0.018	0.009	0.049	0.026
C-70	59.83	58.97	12.12	10.17	23.33	24.27	0.89	0.85	1.78	2.13	0.78	0.68	0.012	0.013	1.70	1.02	0.020	0.010	0.041	0.028
C-71	44.55	48.57	4.00	4.46	30.33	37.49	1.08	1.24	2.37	3.57	0.83	0.71	0.012	0.009	3.92	1.96	0.019	0.022	0.053	0.033
C-72	39.51	46.69	4.00	4.12	33.08	36.69	1.40	0.92	6.40	6.61	1.22	1.19	0.015	0.014	2.75	1.28	0.033	0.045	0.048	0.035
C-73	49.85	56.83	6.95	6.93	24.59	31.12	0.89	0.91	0.85	1.02	0.68	0.82	0.006	0.006	2.95	1.74	0.014	0.010	0.050	0.031
C-74	57.37	54.78	6.30	7.44	25.56	30.85	0.94	1.17	0.73	0.85	0.66	0.85	0.007	0.006	3.08	1.81	0.009	0.018	0.051	0.033
C-75	54.50	50.78	5.82	5.60	29.29	36.31	1.10	0.85	0.78	0.71	0.80	0.99	0.006	0.004	2.83	1.77	0.055	0.032	0.097	0.033
C-76	54.50	55.43	5.49	5.80	29.29	32.95	1.03	0.86	0.48	0.43	0.77	0.96	0.005	0.005	2.31	1.55	0.060	0.025	0.109	0.035
C-77	51.41	53.63	5.12	4.96	27.16	34.82	1.15	0.88	0.55	0.54	0.66	0.81	0.006	0.005	2.78	1.76	0.055	0.037	0.120	0.039
C-78	49.96	55.10	8.20	8.10	27.15	29.19	0.98	0.94	0.44	0.43	0.82	0.80	0.004	0.005	3.06	2.18	0.055	0.028	0.108	0.033
C-80	61.14	62.71	3.02	3.88	22.16	28.73	1.12	1.06	0.60	0.68	0.34	0.49	0.003	0.005	2.23	1.06	0.025	0.021	1.039	0.025
C-81	55.31	57.64	3.02	4.61	19.74	31.79	1.43	1.34	0.60	0.78	0.31	0.56	0.004	0.007	2.58	1.12	0.042	0.033	0.042	0.025
C-82	59.41	57.25	6.30	7.25	23.61	29.01	0.93	0.77	0.77	1.09	0.65	0.88	0.005	0.006	2.30	1.75	0.034	0.054	0.071	0.032
C-83	66.54	59.36	2.94	3.29	26.28	29.78	1.25	1.06	1.23	1.59	0.74	0.85	0.013	0.014	2.55	1.59	0.025	0.033	0.048	0.028
C-84	66.54	62.93	2.13	2.39	23.96	28.69	1.17	1.41	0.56	0.76	0.41	0.54	0.004	0.005	2.17	1.52	0.019	0.027	0.048	0.028
C-85	50.41	58.61	6.30	6.39	29.10	23.33	1.17	1.38	1.10	1.94	0.74	0.56	0.019	0.016	5.27	3.33	0.040	0.136	0.076	0.032
C-86	43.56	42.72	10.96	12.71	23.62	34.39	0.88	0.76	0.65	0.91	0.72	0.91	0.008	0.008	4.84	3.18	0.038	0.043	0.065	0.029
C-88	32.67	34.47	22.00	31.84	19.06	22.63	0.86	0.78	0.71	2.11	0.52	0.26	0.008	..	6.31	3.15	0.033	0.061	0.056	0.031
C-89	36.15	46.56	8.89	10.93	22.93	29.06	0.94	1.25	0.82	2.18	0.64	0.62	0.016	0.012	7.50	3.43	0.042	0.058	0.064	0.068
C-90	41.06	33.63	14.20	22.04	27.16	31.81	1.24	1.13	1.35	1.81	0.71	0.45	0.033	0.021	6.13	2.49	0.047	0.063	0.067	0.039
C-91	45.85	52.13	15.69	16.35	23.54	23.22	0.93	0.82	2.31	1.65	0.92	0.82	0.038	0.027	3.43	1.54	0.037	0.084	0.045	0.084
C-92	38.86	32.69	15.70	24.77	25.62	30.39	0.84	0.76	1.00	1.53	0.76	0.51	0.019	0.013	7.91	2.80	0.042	0.071	0.057	0.044
C-93	42.51	37.01	10.65	11.57	27.16	34.38	1.11	1.22	7.38	6.66	1.72	1.05	0.025	0.018	7.19	1.92	0.031	0.019	0.044	0.030
C-94	39.62	39.38	12.45	11.38	27.16	31.84	1.40	1.38	9.40	9.53	1.84	1.07	0.025	0.020	4.46	1.69	0.029	0.025	0.046	0.031
C-95	41.78	38.27	15.22	13.79	29.27	35.68	1.71	1.37	5.37	5.34	1.25	0.86	0.020	0.017	3.79	1.55	0.032	0.027	0.049	0.031
C-96	45.75	36.00	7.21	10.66	32.12	42.33	1.22	1.33	2.21	2.66	0.83	0.67	0.007	...	8.33	..	0.043	0.085	0.054	0.030
C-97	39.62	34.81	8.22	9.99	37.10	42.96	1.62	1.48	2.24	2.59	1.01	0.74	0.007	...	8.88	..	0.047	0.073	0.049	0.028
C-98	47.60	37.09	5.85	6.45	28.75	33.87	1.15	0.97	2.02	2.25	0.76	0.32	0.007	...	16.12	4.19	0.042	0.039	0.049	0.029

purity should be used as standards until such analyses are available.

Table I also lists the analysis of the coal calculated from the average spectrographic analysis of the ash. Six spectrographic analyses of C-5 coal were made, using C-5 ash as a standard. The agreement between the analyses is very good.

The sulfur in the ash cannot be determined by means of the spectrograph. A certain amount of sulfur remains in the ash and should be accounted for in high-calcium coals. The quantity of sulfur which remains in the ash is dependent on the rate of heating and the maximum temperature attained in the ashing process. If the ashing process is carried out at a carefully controlled temperature of 740° C. the sulfur remaining in the ash is equal to or slightly less than the quantity of sulfur necessary to convert all the calcium to calcium sulfate. The sulfur trioxide can be estimated from the calcium content with a fair degree of accuracy from calcium oxide–sulfur trioxide curves obtained from chemical analyses. It is planned to publish a paper on this subject at a later date.

The size of the sample taken for analysis is only 0.005 that used in the usual burning method. Because of this fact, the sample must be ground much finer. Samples ground to 200-mesh and carefully mixed are suitable in all cases, and in specific instances, 60-mesh coal is fine enough. Especially would this be true of production analyses.

The high temperatures encountered in the direct current arc, 6000° to 7000° C., melt the ash in the electrode. This liquid vaporizes into the arc. Occasionally the liquid boils so rapidly that some of the liquid flies out of the electrode and may be caught in a porcelain dish or paper placed under the electrode. These particles look like tiny marbles of various colors when viewed under the microscope. The operator can usually see sparks flying out of the arc when the liquid flies out. Ashes high in iron fly out more often when arced than any other type. The coals seldom fly out of the electrodes in this manner. Mixing the sample of ash with high-purity electrode carbon appears to prevent splattering of large fused particles. However, the combustion of the fine carbon when the arc first strikes causes particles of sample to be carried through the arc without being excited. This statement also applies to coals that are not completely ashed in the electrode before arcing. Maximum accuracy in this respect is obtained by not having fine carbon particles in the sample while arcing. In the occasional cases where splattering occurs, it is necessary to repeat the analysis.

A recent change in procedure is expected to increase the accuracy materially. It had been the practice to photograph the standard at the top of the plate, followed by the four unknowns. The standard is now photographed third, in the middle of the plate. This procedure tends to balance out differences in the emulsion on the plate and physical changes in the laboratory which occur while five spectra are being photographed.

The weighings for this work were made on a balance with a sensitivity of 0.02 mg. The 5 mg. were weighed out to ±0.05 mg. The analytical method is now sufficiently accurate to warrant the use of a more sensitive balance.

TIME SCHEDULE

The analyses in Table II required slightly more than 1-man-hour per analysis, which can be shortened possibly to 30 minutes in a routine setup. Two men should be able to turn out an analysis in 15 minutes. It should be possible to make data similar to that in Table II, including a calculation of the fusion temperature of the ash, available 20 minutes behind the tipple operations. If the sampling is made at a point 20 minutes ahead of the washing or tipple operations, the analytical data could be made available to the operator ahead of these operations.

SUMMARY

The total energy method consists of photographing separately on a spectrographic plate the light emitted in a direct current arc by the complete excitation of 5 mg. of sample and 5 mg. of a standard of known composition. The optical densities of the lines on this plate are measured, and from these the concentrations of the elements in the sample are calculated.

The ash content of coals can be determined spectrographically on the coal in a fraction of the time required for the customary ashing method at 740 °C. with comparable accuracies.

At the same time the composition of the mineral matter in the coal in terms of the metallic oxides is determined as a part of this spectrographic method. These data can be used for any purpose requiring the composition of ash, particularly to estimate the fusibility of the ash.

ACKNOWLEDGMENT

Sample cuts of coals C-66 to C-98 were graciously supplied by H. M. Cooper and R. F. Abernethy, Central Experiment Station, United States Bureau of Mines, Pittsburgh, Pa., who also supplied their excellent proximate and ultimate analyses of these coals. The authors are deeply grateful for their kind cooperation.

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Conductometric Method for Determination of Carbon in Steel

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An apparatus is described for determining small amounts of carbon in steels by measuring the change in conductivity of a barium hydroxide solution. Results obtained with this apparatus compare favorably with those obtained by the more time-consuming combustion and gas-fractionating apparatus.

IN THE analytical control of steels and certain ferroalloys, carbon is the element most frequently determined. In the standard method, the sample is burned in oxygen and the carbon dioxide is absorbed in a weighed bulb containing Ascarite (*1*). For most steels and ferroalloys this method is rapid, accurate, and subject to the minimum of interference.

Within the past few years, considerable interest has developed in the properties of stainless steels with carbon contents <0.03% and in 0.03% maximum ferrochromium to produce such steels. The authors' experience with the standard combustion method led them to seek a more precise means for determining these low percentages of carbon. With the present furnaces and combustion tubes, the amount of sample that can be burned conveniently is 2 to 3 grams. With a 3-gram sample each milligram increase in weight of the absorption tube is equivalent to nearly 0.01% carbon. Even with counterpoises and other precautions it is difficult to weigh an absorption bulb of approximately 200 grams consistently within ±0.2 mg., and because each bulb must be weighed twice, it is possible thus to introduce an error of at least 0.003% from weighing alone.

Because any new method must have the rapidity and, if possible, the simplicity of the present standard apparatus, titration, bleaching of indicators, and pH measurement, as well as microabsorption bulbs, were ruled out in favor of a study of the change in conductivity of a barium hydroxide solution.

Several articles have described the determination of carbon or carbon dioxide by measuring the change in conductivity of a barium hydroxide solution as it absorbs carbon dioxide (*5*). The earliest work was by Cain and Maxwell (*2*) who developed the method for from 0.2 to 1.0% carbon. Their apparatus was cumbersome and, although commercially available, was never widely used. After the authors had started their study, a paper by Ericsson (*3*) appeared. He further developed Cain and Maxwell's procedure and obtained results with a precision of ±0.0005% carbon on steels with less than 0.05% carbon. The authors' apparatus was constructed before Ericsson's paper was available, but they have since modified their electrodes so that they are similar to his. With his absorber, 15 to 20 minutes were required for each sample. The authors have designed an absorber that can be used at higher rates of oxygen flow and have introduced a number of modifications.

APPARATUS

For the determination of carbon in high-chromium alloys, a Globar combustion furnace is essential. Because the layout of carbon combustion trains is standardized (*1*), only the parts that differ from the standard are described. The essential parts of such a train are: an oxygen purification unit, a combustion tube with furnace, combustion boats, a unit to remove oxides of sulfur, the carbon dioxide absorption unit, and the measuring bridge.

Oxygen Purification Unit. For low carbons, it is necessary to pass the oxygen through a copper oxide furnace and then through Ascarite. The copper oxide furnace is a wire-wound tube furnace operated at 500° C. containing a 1-inch (2.5-cm.) silica tube 15 inches long, filled with wire-form cupric oxide. It is connected to a 1-inch glass tube 12 inches long containing Ascarite and Drierite by means of copper tubing and brass connectors cemented to the tubes with Insalute cement. Oxygen is admitted to the purification furnace by means of an Oxweld R-80 regulator and an Oxweld V-11 valve. A similar 0.25-inch copper tube leads the purified oxygen through a Prest-O-Lite No. 04P82 regulator and a short length of Tygon tubing to the stopper of the combustion tube. By means of a tee, a standard Oxweld 100-pound gage is also connected ahead of the regulator.

Combustion Furnace and Tube. A Burrell Technical Supply Company Model CTA-29 combustion furnace equipped with a thermocouple and operated at 1400° C. is used with a McDaniel combustion tube, 1.25 inches in outside diameter, 1 inch in inside diameter, and 30 inches long. The ends of the combustion tube are cooled by four turns of $^3/_{16}$-inch copper tubing through which tap water circulates. The combustion tube is closed by rubber stoppers equipped with hollow spherical glass heat reflectors. Metal connectors have obvious advantages, which, however, do not outweigh the disadvantage of attaching them to new tubes.

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The combustion tube is empty, but a glass bulb 1.5 inches in diameter, filled with glass wool, is connected to the end of the combustion tube to remove iron oxide dust.

Combustion Boats and Accelerators. Single-trip boats approximately 3.75 inches long, 0.5 inch wide, and $^7/_{16}$ inch high are used without bedding. Boats are ignited previously for several hours, or better, overnight, in a muffle furnace at 1050° to 1100° C. and are removed from this furnace and allowed to cool to approximately 50° to 75° C. just before use.

Bedding material is a frequent source of trouble in low-carbon work. Synthetic aluminum oxide often contains traces of alkali which will pick up carbon dioxide from the air and give it off when heated to the temperature of molten steel; so for low-carbon work bedding material should be eliminated.

Metallic tin is the most desirable flux for low-carbon high-chromium materials. Although certain grades of electrolytic iron are useful for very refractory alloys, with a sufficiently high temperature tin will flux most materials satisfactorily.

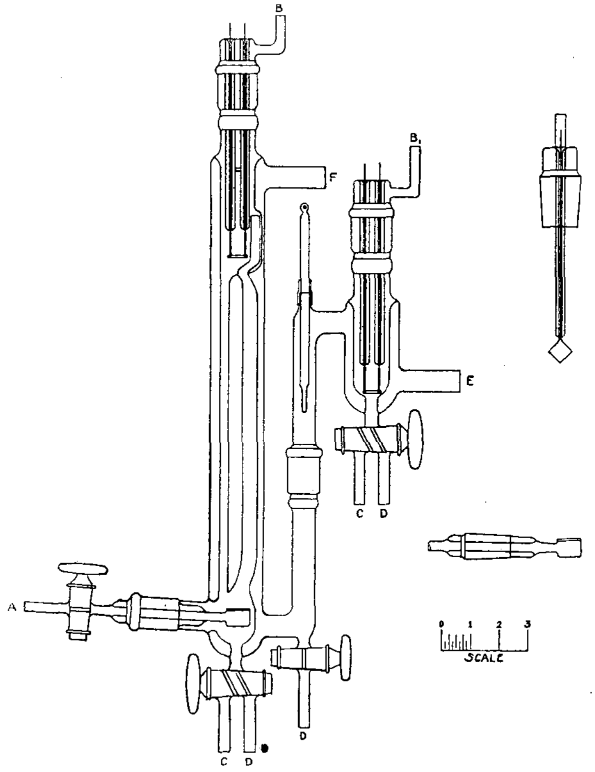

Figure 1. Absorber

Sulfur-Removal Unit. A small vertical furnace with a borosilicate glass tube, 1 inch in diameter by 12 inches filled with Davison Chemical Corporation No. ST 55-104 platinized silica gel and maintained at 440° C., is used to oxidize sulfur dioxide to sulfur trioxide. The sulfur trioxide is fixed in asbestos, that has been impregnated with ferric nitrate and ignited, contained in a second 1 × 12 inch tube connected to the first with an Ŧ joint. This tube also contains layers of glass wool and Drierite and terminates in a mercury-sealed float valve.

Absorption Apparatus. For this train, it was necessary to design a special thermostated absorption cell that would absorb the very small amount of carbon dioxide from the 6 to 8 liters of oxygen passed through it during the burning of the sample and subsequent sweeping of the combustion tube. The absorber evolved from that suggested by Kalina and Joseph (*4*) through several modifications to the form shown in Figure 1, which has been in use for the past 2 years. It was designed for circulation of the barium hydroxide solution on the principle of an air lift and is connected to the sulfur-removal unit through the mercury-sealed valve to prevent solution from flowing back into the furnace.

Basically the absorber consists of a water-jacketed, circulating-type vessel containing the electrodes. The gas stream enters at *A*, which is a 24/40 Ŧ joint terminating in a medium-porosity glass frit and is broken up into small bubbles by the frit. These bubbles pass up the right-hand tube *which terminates* above the electrodes, and the gas finally escapes at *B*, which is protected from the air by a Drechsel bottle containing potassium hydroxide (1 + 9). The entrained barium hydroxide solution pours down over the electrodes, down the left tube, and again contacts the gas stream.

The apparatus is filled to a calibration mark with dilute barium hydroxide solution from a reservoir at *C* and spent solution is drained to the sewer through *D*. The capacity of the system is 65 ml. of barium hydroxide solution.

The reference electrode system shown to the right in Figure 1 is designed so that it can be filled with barium hydroxide solution through *C* or drained through *D*. Outlet *B* is closed by a rubber cap after the cell is filled. Both vessels are surrounded by a common water jacket which is connected to a Miller thermostatic attachment (Carl D. Miller, 327 Salem St., North Andover, Mass.) so that Calgon-inhibited water maintained at 30° ± 0.1° C. enters at *F* and is removed at *E*. The Miller thermostatic attachment consists of an electric heater, a thermostatic switch, a heating chamber, and a circulating pump. By maintaining the solution a few degrees above the ambient room temperature, fluctuations due to temperature are minimized.

The electrodes in the working and reference cells are identical. Each consists of two 15-mm. square platinized electrodes spaced 13 mm. apart, rigidly suspended from a male 34/45 Ŧ glass joint. The glass above the ground portion of the joint is just long enough to permit an 8-mm. tube to be sealed in for exit of the gases. The conductivity cell is fitted to a female 34/45 Ŧ glass joint at the upper end of the absorption vessel. The reference cell is fitted to a female 34/45 Ŧ glass joint at the upper end of a vessel just large enough to accommodate the electrodes. Its exit tube is kept closed when the cell is in place.

When the apparatus is not in operation, the working electrodes are kept in sulfuric-chromic acid mixture, as they tend to become insensitive if left in the barium hydroxide solution. The absorber is rinsed occasionally with dilute hydrochloric acid to remove the deposited barium carbonate from the walls. After rinsing with hydrochloric acid, it must be rinsed with water until every trace of acid is removed.

Inasmuch as only changes in conductivity are to be measured, it is not necessary to have the electrodes in the two cells exactly the same, nor to determine a cell constant for the cells.

Measuring Bridge. The basic circuit of the measuring bridge is given in Figure 2.

It consists of an alternating current bridge containing two 1000-ohm precision resistors, the conductivity cell, the reference cell, a 20-ohm variable resistance, and an 11,111-ohm decade box. The bridge is fed with 60-cycle power using a fixed and a variable transformer to supply 0 to 30 volts.

The bridge output voltage is amplified by a three-stage, high-gain amplifier and fed to a 2-inch cathode ray tube. Any unbalance is indicated by an open loop pattern on the cathode ray tube contrasting to a single straight line when the bridge is balanced. An unbalance of 0.1 ohm will cause a 2-mm. opening in the loop. This amplifier cathode ray tube system of null detection is very much more rugged and less expensive than an alternating current galvanometer and allows the use of standard 60-cycle alternating current rather than the 400- to 1000-cycle oscillator required for the usual headphone null detectors. As can be seen from the wiring diagram and associated parts list, it is constructed of standard radio parts.

Barium Hydroxide Solution. One of the problems in developing this apparatus was preventing the frit in the absorber from becoming plugged with barium carbonate. Another problem was to control the frothing of the barium hydroxide solution at high oxygen flow rates. A third consideration was to secure enough change in the barium hydroxide solution to keep within the sensitivity of the measuring bridge when using a 1-gram sample of a 0.05% carbon steel. For other considerations this was the ideal weight of sample for the purpose, so the barium hydroxide solution was developed to cover the range <0.05% carbon on 1-gram samples and 0.05 to 0.10% carbon (the standard stainless steels) on 0.5-gram samples. With other concentrations of barium hydroxide the range can be extended to other ranges of carbon concentration, but because the measurement is a percentage change, there will be a loss in sensitivity in the lower ranges if, for example, the concentration of barium hydroxide is doubled.

The solution is prepared as follows:

Boil 9 liters of water for 10 minutes, then allow to cool to room temperature while protected from carbon dioxide by means of an Ascarite bulb. Alternatively, the carbon dioxide can be removed by passing carbon dioxide–free air through the solution overnight. Before the solution is cool, add 9 grams of barium hydroxide dihydrate, 1 gram of gelatin, and 5 ml. of 2-ethylhexanol. Allow the solution to cool to room temperature and mix thoroughly. Preserve in a borosilicate glass bottle connected to the absorber, so that this solution can be forced into the absorber with carbon dioxide–free air.

PROCEDURE

Turn on the oxygen and adjust the pressure on the 2-ounce regulator to 10 pounds by means of the R-80 regulator. Fill the absorption vessel to the mark with barium hydroxide solution and adjust the No. O4P82 regulator so that oxygen is flowing through the system at 150 to 200 ml. per minute. The barium hydroxide solution is kept at room temperature and because the jacket on the absorbing vessel is kept a trifle higher, it will take from 1 to 3 minutes to reach equilibrium with oxygen flowing as indicated by constant resistance. Set the decade resistance dials at zero, and null the bridge with the 20-ohm variable resistor, then close the V-11 valve.

Weigh a 1.000-gram sample for material containing 0.05% carbon or less, or a 0.500-gram sample for material containing up to 0.1%. Place the sample in the previously ignited combustion boat. Cover the sample with 1 gram of 30-mesh tin, place in the hot zone of the tube, close the tube, and open the V-11 valve. No change in resistance is indicated for about 1.25 minutes if the oxygen passing through the solution is free from carbon dioxide. When the sample is burned and the carbon dioxide reaches the absorption vessel, the resistance of the barium hydroxide changes, throwing the bridge out of balance. Resistance adjustments can be made on the decade box and the resistance change may be followed. When the resistance becomes constant, usually after about 5 minutes, record it. Empty the absorption vessel, drain it well, close the V-11 valve, and fill again with barium hydroxide. Return the decade to zero resistance, remove the boat from the combustion tube, and replace it with a boat containing the next sample. Open the V-11 valve and proceed with the combustion of the next sample.

CALIBRATION

A calibration curve is prepared by burning fractional weights of a National Bureau of Standards steel and plotting the resist-

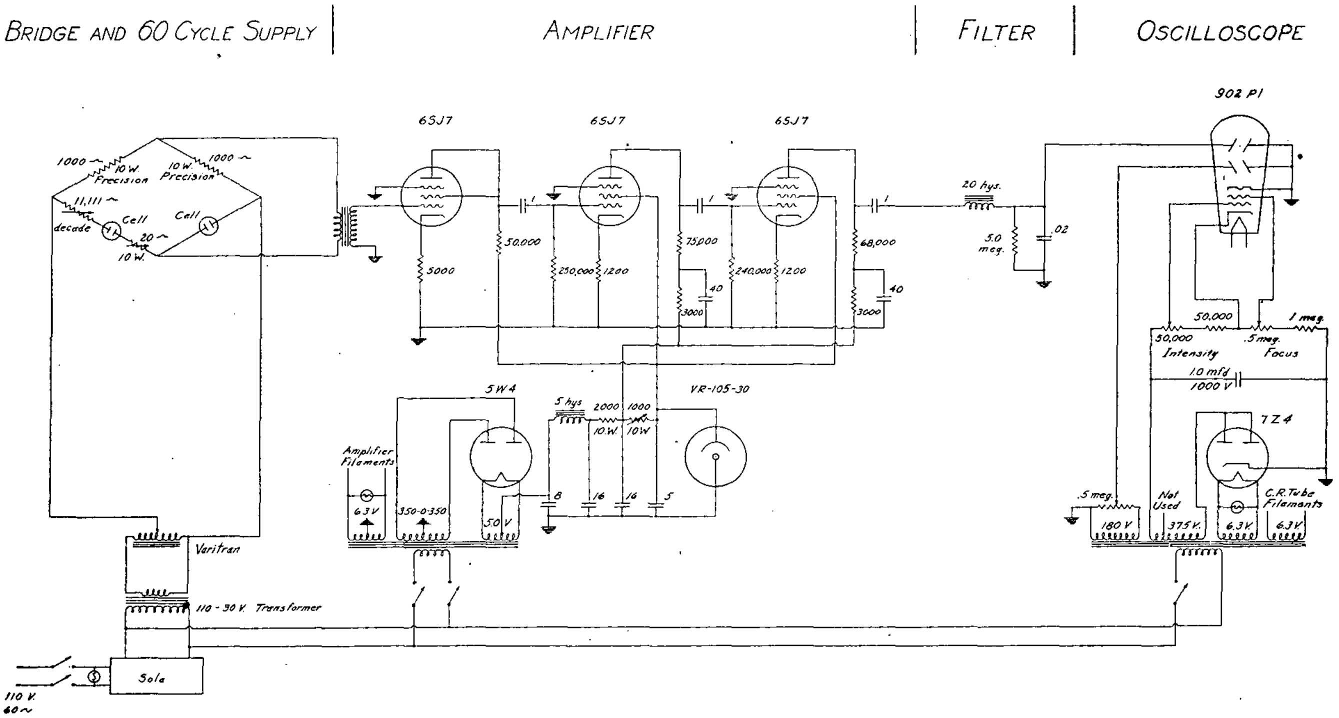

Figure 2. Basic Circuit of Measuring Bridge

	Bridge
2	1000-ohm 10-watt precision resistors (±0.1%)
1	11,111-ohm General Radio decade resistor
1	Model V-2 Varitran
1	20-ohm 10-watt variable resistor
	Amplifier
1	Thordarson type T 15A66 transformer
3	6SJ7 tubes with sockets
1	5000-ohm 1-watt resistor
1	50,000-ohm 1-watt resistor
1	250,000-ohm 1-watt resistor
2	1200-ohm 1-watt resistors
1	75,000-ohm 1-watt resistor
2	3,000-ohm 1-watt resistors
1	240,000-ohm 1-watt resistor
1	68,000-ohm 1-watt resistor
3	0.1-mfd. condensers, 450 volts
2	40-mfd. condensers, 450 volts
1	Stancor P6120 transformer
1	2000-ohm 10-watt resistor
1	1000-ohm 10-watt variable resistor
2	16-Mfd. 450-volt condensers
1	0.5-mfd. 200-volt condenser
1	8-mfd. 450-volt condenser
1	VR-105—30 voltage regulator tube and socket
1	5-henry choke
	Filter
1	5-megohm 1-watt resistor
1	0.02-mfd. condenser
1	20-henry choke
	Oscilloscope
1	902P1 2-inch cathode ray tube and socket
1	7Z4 tube and socket
2	0.5-megohm resistors, 1 watt
1	50,000-ohm resistor, 1 watt
1	1-megohm resistor, 1 watt
1	1-mfd. 1000-volt condenser
1	Power transformer with taps for 375-, 180-, and two 6.3-volt taps
	Miscellaneous
1	Steel cabinet 15 × 22 × 28 inches high
1	8.75-inch rack panel for cabinet
2	5.25-inch rack panels for cabinet
1	7-inch rack panel for cabinet

Necessary hookup wire, panel lights, switches, etc.

Table I. Results Obtained by Conductometric Method on National Bureau of Standards Standards and Other Steels

Sample	Average Value, %	No. of Detns.	Range
N.B.S. 166, cert. 0.028	0.027	12	0.025–0.029
N.B.S. 55b, cert. 0.012	0.011	22	0.0095–0.013
N.B.S. 131, cert. 0.0029	0.0028	6	0.0023–0.0035
Sample A, 0.0047[a]	0.0052	4	0.0051–0.0054
Sample B, 0.0023[b]	0.0022	2	0.0022–0.0023

[a] Average of results of four independent laboratories.
[b] Average of approximately 100 runs by independent laboratories.

ance change against per cent of carbon on the basis of 1-gram samples. Within the range this curve approaches a straight line, so that for most work the daily determination of a single point is sufficient.

DISCUSSION

The absolute accuracy of a method such as this is hard to evaluate. Attempts to determine the carbon dioxide in clean air and to absorb carbon dioxide injected into the oxygen stream from a calibrated gas microburet showed that the absorber and bridge were precise and gave an accurate measure of the amount of carbon dioxide introduced. Yet in the case of low-carbon steels, the variations are greater than can be accounted for unless there are reactions in the combustion zone that are not completely controlled.

For this reason, another method of heating, such as high-frequency induction, would have advantages, for with induced heating any metal present would always be hotter than the surrounding oxide, which is not the case when the metal is heated by conduction and radiation.

A further source of trouble is the equilibrium that is slowly established between the gas stream and the refractory tube. A new tube does not reach equilibrium for several hours, and if the furnace is allowed to cool overnight exposed to air, it may require at least an hour's heating and sweeping to remove the adsorbed carbon dioxide. Perhaps a little more attention on the part of tube manufacturers to securing alkali-free materials might improve this situation. Such an equilibrium exists in all combustion tubes. As is well known, if a cast iron is burned in a tube followed by a low-carbon steel, the carbon indicated for the low-carbon steel will be high. With this train, it is possible to follow with the conductivity bridge the slow removal of this carbon dioxide by the oxygen stream.

Because all these factors can affect the carbon value by contributing to the blank on the train, a standard is usually run several times a day to detect any change in the blank. The normal variation during a day is usually about 0.5 ohm, which corresponds to approximately 0.002% carbon at the 0.07% level. Duplicate determinations on standards are usually within 0.3 ohm. Attempts have been made to run blanks on the closed furnace, and after the first 15 or 20 minutes if the tube was in good condition the indicated blank for 20 minutes was usually less than 0.1 ohm. If the temperature of the absorber is not held constant, or the electrodes become dirty, greater variations will be obtained. About every 6 months the electrodes should be replatinized, as they very slowly lose their sensitivity.

During the past two years, the authors have made several comparisons on low-carbon materials with other laboratories which were using various types of apparatus. Table I lists some of these comparisons and also precision obtainable on replicate analysis of National Bureau of Standards standard samples.

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Colorimetric Determination of Fluoride Ion

Application to Estimation of Monofluophosphate Ion

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The Steiger and Merwin colorimetric procedure for the estimation of fluoride ion has been revised and adapted for the analysis of fluoride ion in the presence of large quantities of phosphate. Extrapolation procedures have been developed for the analysis of fluophosphate compounds capable of being hydrolyzed in acid media to fluoride and phosphate.

THE Steiger and Merwin colorimetric procedure for fluoride ion analysis has been adapted for the analysis of commercial samples of monofluophosphate salts. Because the monofluophosphate ion hydrolyzes in the color medium, a graphical extrapolation is used to obtain the amount of fluoride present as an impurity. The monofluophosphate ion is then determined by hydrolysis to fluoride and orthophosphate, followed by colorimetric determination of fluoride.

Monofluophosphate ion (PO_3F^{--}) does not give the analytical reactions of either fluoride or orthophosphate ions. Although the normal salts of monofluophosphoric acid are similar in nature to the corresponding salts of sulfuric acid, in many cases being isomorphic with the sulfates, the usual procedures applied for gravimetric sulfate determinations are not applicable to monofluophosphate determinations because of an extremely high degree of coprecipitation. Silver monofluophosphate has been precipitated with considerable contamination by the use of 80% alcohol (*4*). After tedious purification of the silver salt, the monofluophosphate ion may be determined by removal of the fluoride ion through distillation with sulfuric or perchloric acid, followed by titration of the distillate with thorium nitrate, according to the procedure of Willard and Winter (*6*).

The purpose of the investigation herein reported was to study the applicability of colorimetric procedures to the determination of monofluophosphate ion. The ease with which this ion is hydrolyzed to fluoride and orthophosphate ions indicated that a suitable colorimetric procedure for fluoride ion might be applied before and after hydrolysis of the monofluophosphate salt in order to find not only the amount of monofluophosphate but also the fluoride impurity. The bleaching effect of fluoride ion on acidic solutions of titanium peroxide complexes was chosen as the most readily adaptable procedure.

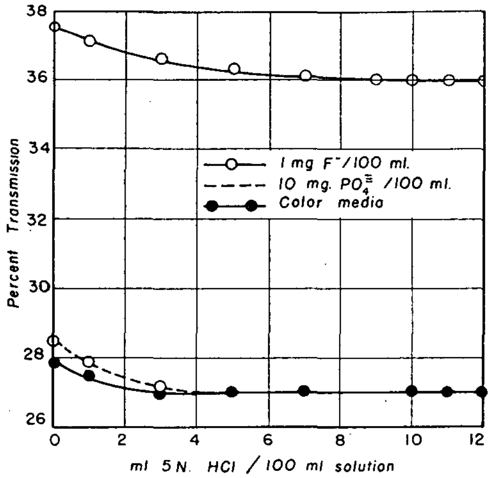

Figure 1. Effect of Acid Concentration

The Steiger (*5*) and Merwin (*2*) procedure for the colorimetric determination of fluoride ion is based on the fact that fluoride ion bleaches the yellow color formed when solutions of titanium salts are treated with hydrogen peroxide in the presence of sulfuric acid. The extent of this bleaching effect was found to be a function of the fluoride ion concentration. These investigators reported that large amounts of phosphates and alkali sulfates also bleached a standard color solution. Increase in temperature or addition of mineral acid was found to increase color intensity, while the presence of ferric or aluminum ion greatly limited the bleaching effect of the fluoride ion. In the final procedure, the amount of acid used in preparation of the sample varied with the approximate fluoride ion concentration.

In a later investigation of the same color system, Dahle (*1*) reported that the bleaching by fluoride ion and the intensity of the original color were both dependent upon the pH of the final solution. In the pH range 1.15 to 1.50, Dahle found that the extent of bleaching was constant within experimental error, although the original color intensity varied. Working in this pH range, he was able to make use of the aluminum ion to restore the color of a portion of the bleached sample without affecting the pH. Thus, the bleaching effect could be evaluated by comparison of two samples of the same pH. No results were reported for any investigation in the pH range below 1.15.

In the most recent investigation, Monnier, Vaucher, and Wenger (*3*) reported the use of solutions having an approximate pH of 0.9. At this pH, no phosphate ion bleaching occurred. The amount of acid used in attaining this pH varied with the amount of phosphate ion present in the sample for analysis. A linear relationship between color intensity and fluoride ion concentration was reported for fluoride ion concentrations from 0.09 to 0.35 mg. per 100 ml. of solution. The system absorption peaks occurred at 400 and 440 mμ and the reported extinction coefficient was approximately 163 liter-gram-ion^{-1} cm.$^{-1}$.

APPARATUS AND REAGENTS

The instrument used in this investigation was a Lumetron Model 402EF balanced bridge-type colorimeter equipped with barrier layer photocells. A multiple-reflection galvanometer of high sensitivity was used to indicate balance in the bridge circuit. A tungsten filament light source with a 400 mμ glass filter was used. Per cent transmittance was determined for 25 ml. of solution contained in a rectangular cell having a 5-cm. light path. The instrument was operated at a sensitivity of 27 units galvanometer deflection for 10 units slide-wire travel. This is slightly higher than recommended, and because no constant voltage device was available during this work, a constant check on sensitivity, light intensity, and bridge balance was necessary. All measurements were made at 23° ± 1° C.

All reagents used were of analytical grade. Solutions were prepared in standard volumetric glassware and stored in glass-stoppered bottles. All solutions were prepared at a temperature of 23° ± 1° C.

Titanium Complex Solution. Twenty-five milliliters of 20% titanous chloride, 100 ml. of concentrated sulfuric acid, and 75 ml. of Superoxol (30% hydrogen peroxide) were added to 600 ml. of water contained in a 1-liter volumetric flask. The resulting mixture was cooled at 23° C. and diluted to 1 liter. This solution gave no evidence of deterioration after 4.5 months. Sixty milliliters of the above solution, diluted to 1 liter, were used as the final color reagent.

Dilution of this color reagent with the solution to be analyzed gave erratic transmittance readings unless an additional amount of hydrogen peroxide was added just before a transmittance reading was taken. The amount of hydrogen peroxide to be added is discussed below.

Standard Fluoride Solution. Purified sodium fluoride was dried at 110° C. to constant weight. A solution containing 1 mg. of fluoride ion per milliliter was prepared by dissolving 1.105 grams of the dried salt in sufficient water to make 500 ml.; 5 ml. of this solution diluted to 500 gave a solution containing 0.01 mg. of fluoride ion per milliliter.

Phosphate Ion Solution. Hydrated disodium phosphate was heated at 100° C. until constant weight was attained. A solution containing 1 mg. of orthophosphate ion per milliliter was prepared by dissolving 0.747 gram of the anhydrous salt in sufficient water to make 500 ml. of solution.

DEVELOPMENT OF PROCEDURE

Effect of Acid Concentration. The effect of added hydrochloric acid concentration upon the percentage transmittance of the titanium peroxide complex is shown in Figure 1. The color intensity of the complex is constant after the addition of approximately 3 ml. of 5 *N* acid. The bleaching effect of 10 mg. of phosphate ion is completely counteracted by the addition of 4 ml. of acid and the bleaching by fluoride ion becomes essentially constant after the addition of 8 to 9 ml. of acid. In view of these results, 10 ml. of 5 *N* hydrochloric acid per 100 ml. of final solution were chosen as the most satisfactory added acid concentration.

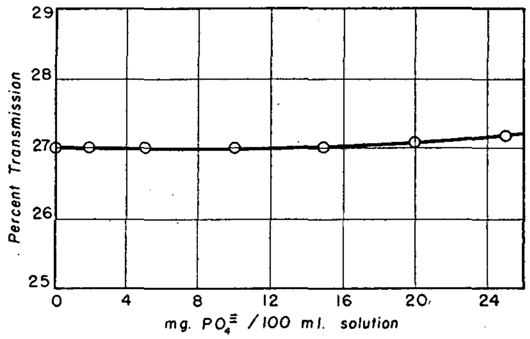

Figure 2. Effect of Phosphate Ion

During the investigation of acid concentration, an attempt was made to substitute sulfuric or nitric acid for hydrochloric. The data obtained were extremely erratic, indicating possible interference by high sulfate and nitrate ion concentrations, although these ions in smaller concentrations apparently do not interfere.

Effect of Phosphate Ion. After choosing 10 ml. of 5 *N* hydrochloric acid per 100 ml. of solution as the optimum added acid concentration, it became necessary to determine the minimum phosphate ion concentration required to bleach the color of the titanium complex. The results of this study are shown in Figure 2. The per cent transmittance did not change for phosphate ion concentrations between 0 and 15 mg. per 100 ml. of solution.

Effect of Hydrogen Peroxide Concentration. Figure 3 presents the data obtained in a series of tests on solutions containing varying amounts of 6% hydrogen peroxide. These data indicate that the amount of added 6% hydrogen peroxide should be at least 8 ml.; 10 ml. were chosen as furnishing sufficient margin of safety to allow use of a prepared solution for several days. Later investigations indicated that such a solution should not be stored longer than 10 days.

Preparation of Calibration Curve. The calibration curve presented in Figure 4 was obtained using 10 ml. each of the color reagent, 5 *N* hydrochloric acid, and 6% hydrogen peroxide. Standard fluoride ion solution was added in the amount required to give the desired concentrations. The curve was run at 23° C., as previously indicated.

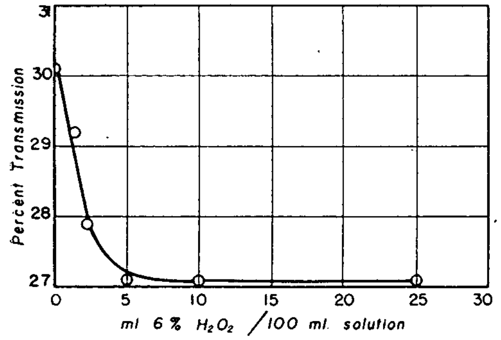

Figure 3. Effect of Hydrogen Peroxide Concentration

Although there appears to be a linear relationship between logarithm of per cent transmittance and fluoride ion concentration from zero to 0.5 mg. of fluoride ion per 100 ml. of solution, this is not actually true. Numerous checks in the range below 0.1 mg. of fluoride ion per 100 ml. plotted on a larger graph, show that the linear relationship begins at about 0.08 mg. per 100 ml. The upper limit of the linear relationship is about 0.4 mg. per 100 ml., with a gradual decrease in slope above this concentration.

Hydrolysis of Monofluophosphate. During the first trial analysis of free fluoride ion in a commercial sample of sodium monofluophosphate, it was found that the monofluophosphate ion hydrolyzed rather rapidly in the color medium. Early attempts to obtain a per cent transmittance reading as soon as possible after mixing of the sample gave nonreproducible results.

Because it appeared impossible to obtain a fluoride ion analysis without some degree of hydrolysis of the monofluophosphate ion, the rate of hydrolysis at different concentrations was investigated. It soon became evident that solutions containing less than 7 mg. of sodium monofluophosphate per 100 ml. of color medium hydrolyzed relatively slowly. Within experimental error, a linear relationship between time and logarithm of fluoride ion concentration was found to exist. Thus, it was possible to prepare graphs of time versus logarithm of fluoride ion concentration, extrapolate to zero time, and obtain a value for the fluoride ion present as impurity in the original sample.

PROCEDURE

Free Fluoride Analysis. A dried, representative sample weighing between 0.4 and 0.7 gram was dissolved in 50 to 100 ml. of water, transferred quantitatively to a 1-liter volumetric flask, and diluted to the mark. A 10-ml. aliquot from this solution was placed in a 100-ml. volumetric flask containing 10 ml. each of the color reagent, 5 *N* hydrochloric acid, and 6% hydrogen peroxide, and 50 ml. of water. The volume was made up to 100 ml. and the sample was vigorously shaken for 2 or 3 minutes. Time was measured from the instant all of the aliquot part was transferred to the color medium, and the per cent transmittance was measured in the usual manner. The time was recorded and successive measurements of per cent transmittance were made at 3-minute intervals with the exact time elapsed recorded for each measurement. The fluoride ion concentration for each recorded time was obtained from Figure 4.

Table I. Analyses of Commercial Samples

Sample No.	Analysis No.	% NaF	% Na_2PO_3F
1	1	7.72	88.7
	2	7.83	88.9
	3	7.76	88.1
	4	7.79	88.3
	5	7.85	88.1
	Av.	7.79	88.4
Av. deviation, %		0.5	0.3
2	1	2.91	92.1
	2	2.92	91.6
	3	3.04[a]	91.9
	4	2.93	91.4
	5	2.91	91.1
	Av.	2.92	91.6
Av. deviation, %		0.3	0.3
3	1	0.931	93.7
	2	0.933	94.5
	3	0.938	93.1
	4	0.945	93.3
	5	0.942	94.1
	Av.	0.938	93.7
Av. deviation, %		0.5	0.5
4	1	1.20	93.4
	2	1.23	93.8
	3	1.20	93.2
	4	1.18	93.7
	5	1.19	94.0
	Av.	1.20	93.6
Av. deviation, %		1.0	0.3
			% $CaPO_3.2H_2O$
5	1		96.8
	2		98.0
	3		97.4
	4		97.5
	5		97.8
	Av.		97.5
Av. deviation, %			0.3

[a] Value not included in average.

In some instances, samples as small as 0.2 gram were handled. The time required for appreciable hydrolysis to occur was considerably lengthened and the slope of the curve obtained was very low, indicating that a relatively small error would be introduced if the first point were taken as representing the fluoride ion present as impurity.

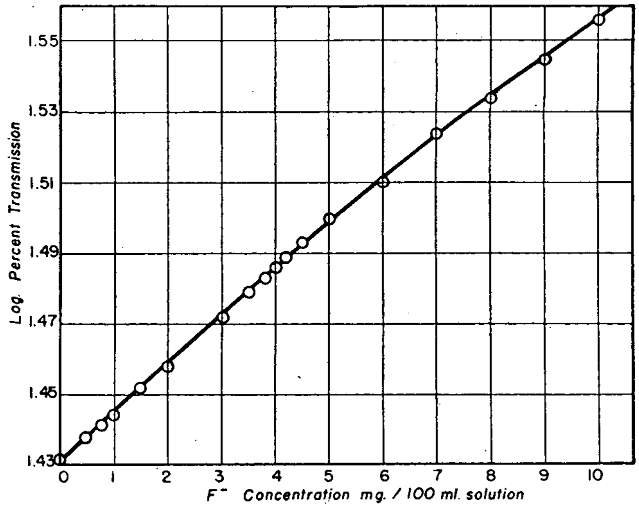

Figure 4. Calibration Curve

If the sample of sodium monofluophosphate weighs more than 0.7 gram, sufficiently small aliquots must be taken for analysis to ensure a final concentration of sodium monofluophosphate below 7 mg. per 100 ml. of color medium. Above this concentration the linear relationship no longer holds because of too rapid hydrolysis.

Monofluophosphate Analysis. A 5-ml. aliquot of the 1-liter sample solution, prepared as for determination of fluoride ion impurity, was transferred to a 100-ml. volumetric flask. Ten milliliters of 5 *N* hydrochloric acid were added and the sample was allowed to stand at room temperature for at least 1 hour. If the original sample weighed less than 0.4 gram, 2 hours were allowed for hydrolysis. The sample was analyzed for fluoride ion impurity during this time interval. After sufficient time had been allowed for complete hydrolysis, 10 ml. each of the color reagent and 6% hydrogen peroxide were added and the total volume was made up to 100 ml. This solution was thoroughly mixed and the per cent transmittance was determined. The total fluoride ion concentration corresponding to this transmittance reading was taken from Figure 4, and was corrected for the amount of impurity and converted to per cent sodium or calcium monofluophosphate. In the analysis of the calcium salt, no attempt was made to determine the fluoride ion impurity, as the salt was not soluble enough in pure water to allow determination in the usual manner.

ANALYTICAL RESULTS

Table I presents the results obtained from the analysis of four commercial samples of sodium monofluophosphate and one sample of the calcium salt furnished by the Ozark-Manhoning Company, Tulsa, Okla. These samples contained only sodium fluoride, sodium hexametaphosphate, and sodium carbonate as impurities.

SUMMARY

The Steiger (*5*) and Merwin (*2*) colorimetric procedure for fluoride ion analysis has been adapted for the analysis of commercial samples of monofluophosphate salts. Because the monofluophosphate ion hydrolyzes in the color medium, a graphical extrapolation has been used to obtain the amount of fluoride ion present in the form of fluoride salts. The monofluophosphate ion has been determined by hydrolysis to fluoride and orthophosphate ions, followed by determination of the fluoride ion and calculation to monofluophosphate.

The limits of concentration to which this method are applicable are 0.1 to 1.0 mg. of fluoride per 100 ml. of final solution.

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Fire Assay and Gravimetric Determination of Rhodium

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Two methods for the determination of rhodium in lead-rhodium buttons are described. These were applied to buttons obtained by the fire assay of salted rhodium ores under various conditions, and slag losses were determined. A method for the determination of rhodium in the presence of lead, zinc, and nickel is reported.

IN THE analysis of ores and concentrates for platinum metals, fire assay procedures are usually recommended for a preliminary separation of the precious metals from the gangue (*4, 8, 12, 15, 16*). Although much work has been published on methods for the separation and determination of the metals following pot assay and cupellation (*2–4, 8, 12, 16*), and some work has been reported dealing with cupellation difficulties (*15, 20*), little attention has been paid to the efficiency of the lead collection of platinum metals in the crucible fusion itself; it is generally accepted as satisfactory by analogy with gold and silver.

A survey of the literature revealed the following reports on the lead collection of the precious metals. Fulton and Sharwood (*8*) reported combined slag and cupel losses up to 3% for gold and 5% for silver in copper-bearing ores, and slag losses up to 1% for each metal in simple ores. Shepard and Dietrich (*16*) state that slag losses should be less than 0.5%. Adam and Westwood say that the platinum metals are not collected as well as gold and silver (*1*). Seath and Beamish (*15*) found losses up to 15% in the recovery of precious metals (chiefly platinum and palladium) from nickel ore concentrates. Thiers, Graydon, and Beamish (*19*) recorded ruthenium losses up to 30% under various assaying conditions.

The present investigation deals with the collection of rhodium, and forms part of a systematic examination of the analytical chemistry of the platinum metals.

DETERMINATION OF RHODIUM IN ASSAY BUTTONS

Cupellation of lead-rhodium alloys is unsatisfactory (*20*), as lead cannot be completely removed because of the high melting points of rhodium-rich alloys. The customary addition of silver fails, as rhodium is insoluble in silver (*6*). Rhodium may be lost during cupellation because of oxidation (*1, 11*) and subsequent mechanical loss of oxide particles. Consequently, wet methods of button analysis were used.

Nitric acid parting of lead-rhodium alloys is recommended by a number of authors, and procedures are given for the determination of rhodium following such parting (*14, 17*). In the course of this parting, a variable amount of rhodium is dissolved with the lead, while the residue consists of rhodium contaminated with small quantities of lead. Lead is precipitated from the parting solution as the sulfate (*13, 23*). The parting residue is dissolved in sulfuric acid (*22*) and combined with the filtrate from the lead precipitation, and rhodium is precipitated as the sulfide for determination as the metal.

There are several weaknesses in such a procedure:

Lead sulfate is appreciably soluble under the conditions of its precipitation, so that separation of lead from the rhodium is incomplete.

Precipitation of large amounts of lead sulfate from a solution containing small quantities of rhodium involves the occlusion of considerable rhodium, and a double precipitation is a very unsatisfactory operation with large amounts of lead.

Precipitation of rhodium sulfide from solutions of the sulfate is incomplete.

Various reagents for rhodium were tested in an effort to find a satisfactory means of determining rhodium in the presence of milligram quantities of lead. It was found that thiobarbituric acid (*5*) did not precipitate lead from solutions of the chloride or nitrate, but that the reagent did not precipitate rhodium quantitatively from yellow sulfate solutions; as in the sulfide method, rhodium had to be present as the pink chloride complex. The conventional method for converting the sulfate to the chloride is to boil with concentrated hydrochloric acid (*9*, *10*). This was unsatisfactory with thiobarbituric acid, and has been reported to give inconsistent results with hydrogen sulfide (*17*).

THIOBARBITURIC ACID PRECIPITATION OF RHODIUM FROM SULFURIC ACID SOLUTION

Fuming to a very small volume (<0.5 ml.) of sulfuric acid solution, followed by treatment with hydrochloric acid, gave good precipitations with thiobarbituric acid, but during the fuming a precipitate, insoluble in either water or hydrochloric acid, was frequently formed. It was found that addition of sodium chloride to the sulfuric acid solution, evaporation to crystals, and taking up in water gave a solution from which rhodium could be quantitatively precipitated with the reagent. The sodium chloride neutralized the sulfuric acid by liberation of hydrochloric acid, and supplied excess chloride ions for the formation of the rhodium complex.

Results of a number of determinations were high, however, and microscopic examination of ignited precipitates showed white cubic crystals, evidently sodium chloride, even when large volumes of wash solution had been used. Substitution of ammonium chloride for sodium chloride eliminated this difficulty, and also reduced the volume of wash solution required.

In later work when determinations were carried out following the use of nitric-sulfuric acid mixtures for the destruction of organic matter, it was found that unless the solution were strongly fumed following the last addition of nitric acid incomplete precipitation occurred. This was apparently due to the formation and persistence of nitrite complexes of rhodium (*21*). The substitution of 30% hydrogen peroxide for nitric acid as an oxidizing agent eliminated the possibility of losses on this account.

Reagents. Standard rhodium solutions were prepared by dissolving weighed amounts of sodium rhodium chloride (reported as 21.3% rhodium) in sufficient concentrated hydrochloric acid and water to give a final hydrochloric acid concentration of 0.3 *N*. As the composition of the salt did not correspond to that of any known compound, the rhodium content of each solution was checked by thiobarbituric acid determinations, using aliquot portions of the solution. Rhodium sponge used was obtained from Johnson, Matthey, and Mallory. Both the metal and the salt were examined spectrographically, and neither showed the presence of metal impurities.

Procedures. Aliquot portions of standard rhodium solutions (chloride) were fumed with 5 ml. of concentrated sulfuric acid to convert the rhodium to the sulfate, and the following procedures were carried out. Hot distilled water was used as a wash liquid.

A. The sulfuric acid solutions of rhodium were fumed to a volume of 5 ml., 50 ml. of 20% ammonium chloride solution were added to each, and the solutions were evaporated until crystallization took place. During this treatment the color of the solutions changed from the yellow of the sulfate to the pink of the chloride. Each sample was taken up in water, filtered, and diluted to 200 ml. The final solutions had a pH of 0.9 to 1.1.

B. Each rhodium chloride solution was made up to 200 ml. in a 400-ml. beaker, and if not already acid was acidified with 0.5 ml. of concentrated hydrochloric acid. It was heated to boiling, 10 ml. of a 1.4% weight/volume ethyl alcohol solution of thiobarbituric acid were added, and heating was continued for 2 hours. During this time the volume of the solution was kept constant by additions of hot distilled water. Bumping was avoided by the addition of a small piece of ashless filter pulp. At the end of the digestion time the mixture was filtered through a 7-cm. No. 42 Whatman paper and the precipitate was washed. The precipitate and paper were transferred to a Coors 000 crucible, dried under an infrared lamp, ignited in air at 850° C. for 15 minutes, and reduced in hydrogen for 10 minutes. The crucible was cooled in hydrogen for 5 minutes, cooled in nitrogen for 5 minutes, placed in a desiccator for 10 minutes, placed on the balance, tared, and at the end of 10 minutes, weighed. Saturated solutions of calcium nitrate were kept in the desiccator and in the balance case to maintain constant humidity (*18*). Results of a number of determinations are recorded in Table I.

Table I. Determination of Rhodium in Sulfuric Acid Solutions by Means of Thiobarbituric Acid

Rh Taken *Mg.*	Rh Found *Mg.*	Salt Used	Volume of Wash Water *Ml.*	Error *Mg.*
10.68	11.78	NaCl	20	+1.10
10.68	13.83	NaCl	20	+3.15
10.68	10.60	NaCl	150	−0.08
10.68	10.76	NaCl	150	+0.08
10.68	10.70	NaCl	150	+0.02
10.68	10.84	NaCl	150	+0.16
10.68	10.62	NH_4Cl	50	−0.06
10.68	10.67	NH_4Cl	50	−0.01
10.68	10.63	NH_4Cl	50	−0.05
10.68	10.65	NH_4Cl	50	−0.03
10.68	10.72	NH_4Cl	50	+0.04

Table II. Determination of Rhodium in Presence of Lead and Other Base Metals

Base Metal and Amount *Mg.*	Volume of 10% NH_4Ac Wash Soln. *Ml.*	Rh Taken *Mg.*	Rh Found *Mg.*	Error *Mg.*
100 Zn, 100 Ni	50	10.40	10.44	+0.04
100 Zn, 100 Ni	50	10.40	10.42	+0.02
100 Zn, 100 Ni	50	10.40	10.36	−0.04
100 Zn, 100 Ni	50	10.40	10.43	+0.03
100 Zn	50	10.40	10.39	−0.01
100 Ni	50	10.40	10.42	+0.02
1 Pb	100	11.17	11.18	+0.01
1 Pb	100	11.17	11.19	+0.02
1 Pb	100	11.17	11.26	+0.09
5 Pb	150	11.17	11.63	+0.46
5 Pb	150	11.17	11.74	+0.57
10 Pb	200	11.17	12.03	+0.86
10 Pb	200	11.17	12.06	+0.89
10 Pb	500	10.40	10.76	+0.36
10 Pb[a]	50 each time	10.40	10.41	+0.01
10 Pb[a]	50 each time	10.40	10.42	+0.02
10 Pb[a]	50 each time	11.17	11.15	−0.02
10 Pb[a]	50 each time	11.17	11.11	−0.06

[a] Double precipitations.

DETERMINATION OF RHODIUM IN PRESENCE OF LEAD AND OTHER BASE METALS

Samples containing aliquot portions of standard rhodium solutions and various amounts of lead and other base metals (all as their chlorides) were treated by Procedure B for the determination of rhodium. Double precipitations were carried out by treating the first precipitate and paper with 5 ml. of concentrated sulfuric acid and successive amounts of 30% hydrogen peroxide to destroy organic matter and dissolve the rhodium. The resulting sulfuric acid solutions were treated by Procedures A and B for the determination of rhodium. In all cases a hot 10% ammonium acetate solution was used as a wash liquid. The results of a number of determinations are given in Table II.

NITRIC ACID PARTING OF LEAD-RHODIUM BUTTONS

A number of lead-rhodium buttons were parted with nitric acid, and the residues were dissolved in concentrated sulfuric acid. In place of the objectionable lead sulfate precipitation, a zinc reduction method was used to recover rhodium from the parting solution (*14*, *17*). Although slight losses occurred with this method, there was less interference by copper, which is frequently found in assay buttons. The incomplete recovery of rhodium was thought to be due to the stability of nitrite-type complexes of rhodium formed in the solution (*21*). It has been reported that ruthenium cannot be recovered completely from nitric acid solutions by zinc reduction methods (*19*), and the formation of stable complexes with the oxides of nitrogen is one of the strong family traits of the platinum metals.

Synthetic lead-rhodium buttons were prepared by fusing weighed amounts of rhodium with 25 grams of lead foil in a graphite crucible. Each button was treated with 125 ml. of 1 to 4 nitric acid on a steam bath until visible action ceased. A clear solution and a black residue resulted. The mixture was filtered and the residue washed with 50 ml. of hot 10% ammonium acetate solution. The residue and filter paper were treated with 5 ml. of concentrated sulfuric acid and successive portions of 30% hydrogen peroxide to destroy organic matter and dissolve the rhodium. The solution so obtained was treated by Procedures A and B for the determination of rhodium. (In this and subsequent work double precipitations were used to avoid lead contamination, and washing was carried out with 50 ml. of hot 10% ammonium acetate solution.)

To the combined filtrate and washings from the parting solution 100 mg. of zinc dust in aqueous suspension were added, and the mixture was heated on a steam bath for 6 to 8 hours, then filtered. The filtrate was retreated with 100 mg. of zinc in suspension and the precipitate was examined spectrographically. In no case was there any indication of rhodium in the second treatment. The precipitate and filter paper from the first zinc reduction were treated by Procedures A and B for the determination of rhodium. The results of a number of determinations are given in Table III.

PERCHLORIC ACID PARTING OF LEAD-RHODIUM BUTTONS

Perchloric acid has been used in the analysis of lead-ruthenium buttons (*19*), and offered advantages over nitric acid in avoiding nitrite complexes. Preliminary investigations showed that when 25-gram buttons containing ca. 10 mg. of rhodium were treated with 50 ml. of hot 72% perchloric acid, lead and some of the rhodium dissolved with the evolution of hydrogen. When visible action had ceased, a fine black residue consisting of rhodium and small amounts of lead remained. On continued heating this residue dissolved slowly, and the solution became yellow.

At low temperatures with less active metals perchloric acid acts as a strong, nonoxidizing acid, as indicated by the evolution of hydrogen when it dissolves lead. When heated to fuming it may become oxidizing (cf. sulfuric acid) and its reactions become violent. Lead fuses and may become red hot; it assumes a spheroid shape (cf. sodium with water). Even when cold, concentrated perchloric acid reacts very violently with powdered zinc and similar active metals. Consequently, the greatest care is necessary not to overheat solutions used in the parting procedure. In dissolving buttons heat was applied only when the reaction slowed down, and no "incidents" occurred in many determinations carried out over a 2-year period.

A number of synthetic buttons were prepared by fusing weighed amounts of rhodium metal with 25 grams of lead foil in a graphite crucible. Then 35 ml. of 72% perchloric acid were added to each in a 500-ml. round-bottomed flask, and the flask was heated over a small burner. As soon as effervescence ceased the flask was cooled, and 100 ml. of distilled water were added to dissolve the lead perchlorate. The mixture was filtered and the precipitate washed with 50 ml. of hot distilled water. The combined filtrate and washings were treated with 500 mg. of zinc dust in aqueous suspension and left overnight on a steam bath. The mixture was filtered, the precipitate was washed with 50 ml. of hot distilled water, and the combined filtrate and washings were retreated with 500 mg. of zinc. The three precipitates and papers were treated with 5 ml. of concentrated sulfuric acid and successive portions of 30% hydrogen peroxide to destroy organic matter and dissolve the rhodium. After cooling, this solution was diluted cautiously with 50 ml. of water, cooled again, and filtered. If an appreciable amount of lead sulfate was present, the precipitate and paper were treated again with 5 ml. of concentrated sulfuric acid and hydrogen peroxide to recover occluded rhodium and destroy organic matter. After fuming and cooling, 50 ml. of water were added, and the mixture was cooled and filtered. The two filtrates were combined, and the solution was fumed to 5-ml. volume. All evaporations and "fumings" were carried out in 500-ml. flasks to avoid losses by spattering. The rhodium sulfate solution was treated by Procedures A and B to determine rhodium. The results of a number of determinations are given in Table IV.

DISCUSSION

Of the two parting procedures, the perchloric acid method gave the better results—99 to 101% recovery of rhodium as compared to 97 to 98% with the nitric acid method. However, copper, which occurs in buttons obtained from copper-bearing ores, interferes in the perchloric acid method, as it precipitates on treatment with zinc, and coprecipitates with rhodium in the thiobarbituric acid determination. In the nitric acid procedure copper dissolves in the parting solution, but is precipitated by zinc. A good recovery of rhodium with low copper contamination can be obtained by continued heating of the zinc reduction precipitate in contact with the nitric acid parting solution until the red copper precipitate just disappears. A black rhodium residue is left.

The above methods were satisfactory for the examination of the distribution of rhodium during the fire assay of various types of ores.

RECOVERY OF RHODIUM FROM ORES BY FIRE ASSAY

Ores of several types were salted with known amounts of rhodium, and assayed. The slags were reassayed, and the buttons obtained were treated by one of the parting procedures described above to determine their rhodium content.

Siliceous ores. To test the recovery of rhodium from siliceous ores, the following ore-flux mixture was made up and thoroughly mixed:

Silica, g.	150	Litharge, g.	530
Soda, g.	180	Flour, g.	2.9
Lime, g.	25		

Fusions of this flux gave slags of sesquisilicate composition (*4*, *16*). Ninety-one grams of the flux gave a 27- to 28-gram button.

Samples of powdered rhodium metal were ignited, reduced, and weighed by the methods used in determinations, rolled in a sheet of cellophane with 70 grams of the above flux, and placed in 20-ounce assay pots. The cellophane was cleaned by rolling 21 grams of the flux in it, and adding the flux to the sample as a cover. The pots were placed in a furnace at 1600° F. and the temperature was raised to 2200° F. over a period of 1 hour, at the end of which time the samples were poured into the usual conical steel molds. (The furnace used was a Williams and Wilson 25-cycle 15 kv.-amp. Globar-type assay furnace.) After cooling, the buttons were broken away from the slag and treated by one of the above parting methods for the determination of rhodium. The slags were ground, 2.9 grams of flour with litharge sufficient to replace the lead lost to the first button were added, and the mixture was rolled and reassayed in the original pot. The button so obtained was treated to determine its rhodium content. A second reassay of the slag in no case led to the recovery of rhodium.

Sulfide ores. The platinum metals are frequently associated with nickel in sulfide ores. To test the recovery of rhodium from such ores, a nickel-copper-iron ore from Northern Ontario was used. Its composition was approximately 3% nickel, 3% copper, 25% iron, and 45% silica, and it had a reducing power of 3.7 (*4*, *16*). Assays of the natural ore showed less than 0.05 mg. of total platinum metals per 0.5 assay ton. Two types of treatment are commonly used in assaying sulfide ores for platinum metals. One involves a preroasting which converts the sulfides to oxides, after which the ore is treated as an oxide ore. In the second, potassium nitrate is added to the flux and sulfide is converted to sulfate in the course of the fusion.

PREROASTING METHOD. One hundred grams of the ore were roasted in a shallow fire-clay dish at a temperature of 1400° F.

Table III. Nitric Acid Parting of Lead-Rhodium Buttons

Rh Taken Mg.	Rhodium Recovered: From residue Mg.	Rhodium Recovered: From solution Mg.	Total Mg.	Error Mg.
10.55	10.01	0.20	10.21	−0.34
11.09	10.49	0.22	10.71	−0.38
11.02	10.61	0.26	10.87	−0.15
11.02	10.53	0.22	10.75	−0.27

Table IV. Perchloric Acid Parting of Lead-Rhodium Buttons

Rh Taken Mg.	Rh Recovered Mg.	Error Mg.
6.00	5.91	−0.09
7.98	7.90	−0.08
9.60	9.47	−0.13
10.43	10.30	−0.13
10.48	10.49	+0.01
11.13	11.21	+0.08
13.11	13.26	+0.15

Table V. Recovery of Rhodium from Ores by Fire Assay

No.	Ore Type	Slag Composition	Button Weights 1st G.	Button Weights 2nd G.	Parting Method	Rh Added Mg.	Rh Recovered 1st button Mg.	Rh Recovered 2nd button Mg.	Rh Recovered Total Mg.	Error Mg.
1	Silica	Sesquisilicate	27	30	$HClO_4$	12.64	12.18	0.15	12.33	−0.31
2	Silica	Sesquisilicate	28	29	$HClO_4$	16.26	16.31	0.08	16.39	+0.13
3	Silica	Sesquisilicate	27	27	$HClO_4$	17.69	17.29	0.32	17.61	−0.08
4	Silica	Sesquisilicate	24	21	HNO_3	11.17	10.52	0.22	10.74	−0.43
5	Silica	Sesquisilicate	24	23	HNO_3	11.21	10.60	0.35	10.95	−0.26
6	Silica	Sesquisilicate	23	23	HNO_3	10.71	10.35	0.17	10.52	−0.19
7	Silica	Sesquisilicate	9.6	20	HNO_3	10.68	7.54	2.15	9.69	−0.99
8	Sulfide[a]	Subsilicate	23	25	HNO_3	10.69	10.06	0.44	10.50	−0.19
9	Sulfide[a]	Subsilicate	23	25	HNO_3	10.69	9.51	0.54	10.05	−0.64
10	Sulfide[b]	Subsilicate	20	27	HNO_3	10.69	10.84	0.20	11.04	+0.35
11	Sulfide[b]	Subsilicate	20	26	HNO_3	10.69	10.45	0.15	10.60	−0.09
12	Nickel oxide	Subsilicate	26	20	$HClO_4$	10.69	9.96	0.78	10.74	+0.05
13	Nickel oxide	Subsilicate	29	26	$HClO_4$	10.69	10.21	0.41	10.62	−0.07

[a] Treated by preroasting procedure.
[b] Treated by niter assay procedure.

The weight of the resulting oxide ore was 93.2 grams. Half assay ton samples of the roasted ore were placed in 20-ounce pots which had been glazed with a slag of monosilicate composition, 2-ml. portions of a standard sodium rhodium chloride solution were added to each, and the samples were dried under an infrared lamp. Each was then thoroughly mixed with a subsilicate flux (*16*) made up as follows:

Silica, g.	3	Litharge, g.	125
Soda, g.	14	Flour, g.	2.6

The pots were placed in a furnace at 2200° F. for 40 minutes, and poured. The buttons were broken away from the slag, which was ground and reassayed with added flour and litharge. Owing to the presence of copper, the nitric acid parting procedure was used. Copper contamination in the final rhodium precipitate was estimated spectrographically to be less than 0.10 mg.

NITER ASSAY METHOD. Half assay ton samples of the natural ore were salted with standard rhodium solution in 20-ounce pots, as described for the preroasting method. After drying, each sample was mixed with the following subsilicate flux (*18*):

Silica, g.	1.8	Litharge, g.	125
Soda, g.	17	Niter, g.	10.5

Pots were placed in a furnace at 2200° F. for 30 minutes, and poured. The buttons were broken away from the slag, the salt layer was recovered and set aside for testing, and the slag was ground and reassayed with flour and litharge. Buttons were treated by the nitric acid parting procedure. The salt layer (sodium and potassium sulfates) was dissolved and tested for rhodium with stannous chloride. In no case was rhodium found. The results of a number of determinations by the above methods are shown in Table V.

Nickel ores. Seath and Beamish (*15*) reported that nickel present as the oxide in large quantities caused high losses in the assay of platinum and palladium. As the recoveries obtained with the nickel-copper sulfide ore were of the same order as those obtained with siliceous ores, a synthetic ore with high nickel oxide content was prepared, and salted samples of it were assayed for rhodium.

Half assay ton samples of an ore made up of 1 part of nickel oxide to 5 parts of silica (13% nickel) were salted with standard rhodium solution in glazed 20-ounce pots, dried, and thoroughly mixed with a subsilicate (excess litharge) flux made up as follows:

Soda, g.	15
Litharge, g.	180
Flour, g.	2.6

Assaying and reassaying were carried out as described for the preroasted ore. Buttons were analyzed for rhodium by the perchloric acid parting method. The results of a number of determinations are shown in Table V.

DISCUSSION

The fact that losses for different types of ores and different assay conditions vary over the same range of values suggests that losses may be mechanical rather than chemical in nature. The failure of a second reassay of the slag to recover rhodium not accounted for elsewhere is in agreement with this. Mechanical losses might be due to the incorporation of small particles of rhodium in the glazed walls of the assay pot. The small quantities of rhodium involved prevented the investigation of such losses in the absence of radioisotopes of rhodium. The inefficient collection with a small button is illustrated in determination 7. The low losses for rhodium compared to ruthenium (*19*) can be explained by the difficulty of forming ruthenium alloys with lead, compared to the ease with which rhodium alloys with lead (*6*).

SUMMARY

A procedure for the determination of rhodium in the presence of lead, nickel, and zinc, using thiobarbituric acid, has been developed. A method has been found for converting rhodium sulfate to the chloride preparatory to the thiobarbituric acid determination. An improved procedure for the determination of rhodium in lead buttons by nitric acid parting and a new procedure involving the perchloric acid parting of lead-rhodium buttons have been described.

In the fire assay of rhodium, losses are not constant for given slag compositions and fusion temperatures. These losses can be reduced by reassaying the slag.

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Preparing Cuprammonium Solvent and Cellulose Solutions

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A critical review of the literature suggested the possibility of preparing cuprammonium reagent by dissolving solid cupric hydroxide in ammonium hydroxide and of protecting the cellulose from oxidation during dispersion by the addition of metallic copper or cuprous chloride. This simple method of protection makes possible the use of Erlenmeyer flasks as dissolving vessels instead of more complicated equipment. The following very simple procedure was devised. The cuprammonium reagent was prepared by dissolving solid cupric hydroxide in ammonium hydroxide in an ice bath. The solution of cellulose in cuprammonium was prepared in rubber-stoppered Erlenmeyer flasks by mechanical agitation of the cellulose and the cuprammonium solution, to which cuprous chloride and copper wire had been added. The time of flow of the solution was measured in a viscometer enclosed in a glass jacket thermostated by water pumped from a constant temperature water bath. This procedure saved time, and the results compared favorably with those obtained with a more orthodox method.

THE cuprammonium test is frequently used to estimate the extent of degradation of cellulose, and has been set up in a variety of standard methods (*1, 7, 10, 13, 36, 39*) for use here and abroad. These methods are based upon the investigations of Schweizer (*30*), Ost (*24*), Gibson, Spencer, and McCall (*14*), Joyner (*19*), Farrow and Neale (*12*), Small (*31*), Clibbens and Geake (*6*), and others.

Because the process employed in the standard methods for the preparation of the cuprammonium reagent by bubbling air over copper metal in ammonium hydroxide is tedious and time-consuming, the reagent is usually prepared in large batches and elaborate precautions are taken for storage. The procedures specified in these methods for the nondegradative dispersion of cellulose in the cuprammonium reagent require specialized equipment. These are strong deterrents to the use of the test.

The difficulties involved in the preparation and use of cuprammonium have been extensively recognized, and partially explain the search for other reagents, such as cupri-ethylenediamine (*4, 34*) and dimethyldibenzyl ammonium hydroxide (Triton F) (*4, 22, 26*), following the pioneer work of Traube (*37*) on the substituted amines.

Strauss and Levy (*34*) claim that substituted amine-type solvents offer the advantages of rapid dispersion and slight degradation of cellulose during dispersion. The latter has been questioned by Clibbens (*5*) and the former appears to be true only under certain conditions, as shown by Jolley (*18*).

These substituted amines are rather costly and vary considerably from one batch to another, and the viscosity values obtained with them differ from those obtained with cuprammonium; therefore much confusion would result in the years required to change standard methods.

Rather than a complete change to another method, an attempt to improve the cuprammonium procedure would seem preferable. This article describes a very simple procedure for the preparation of cuprammonium reagent and for the dispersion of cellulose therein, requiring no special equipment. This procedure merely constitutes an amalgamation of techniques already described in the literature, resulting in considerable saving of time and effort without sacrifice of precision.

INVESTIGATION OF TECHNIQUES

The two principal sources of difficulty in the cuprammonium test are the oxidation of cellulose during the dispersion process and the preparation of the reagent.

Avoidance of Oxidative Degradation during Dispersion. Scheller (*28*) has shown that a 10% decrease in the viscosity of a cuprammonium dispersion of cellulose resulted from an amount of added persulfate roughly equivalent to 0.25 ml. of air. Some investigators have sought to avoid the effect of oxygen during dispersion by the use of hydrogen or nitrogen as a displacing gas (*12, 14*). Others (*1, 6, 7, 10, 21, 23, 29, 35, 36*) completely filled the mixing vessel, either the viscometer tube itself, or a bottle, with the reagent.

Entirely different methods of protecting the cellulose against oxidation during dispersion have been described by Scheller (*28*), Doering (*11*), and Battista (*2*). Scheller added cuprous chloride powder, and Doering added copper metal, to the cuprammonium at the outset of the mixing process, with the result that the deteriorative effect of the air present was greatly diminished or entirely eliminated. Doering showed, from viscosity measurement, that the presence of pieces of metallic copper prevented oxidation of the cellulose as effectively as displacing the air with nitrogen. The protection afforded by metallic copper has recently been confirmed by Hisey and Brandon (*16*) for cotton. Scheller showed that 0.1 gram of cuprous chloride in 100 ml. of cuprammonium reagent afforded considerable protection for cotton cellulose; without the cuprous chloride the viscosity values decreased by two thirds (*28*).

These results are of great importance to the test, for they suggest that a very simple technique of mixing, using commonplace equipment, will suffice. A confirmation was desirable, and accordingly experiments were performed using a high-grade cellulose derived from unused cotton fabric.

Rubber-stoppered, 125-ml. Erlenmeyer flasks, charged with 0.231 gram of cellulose (dry weight), 50.0 ml. of cuprammonium reagent, and varying amounts (0 to 0.5 gram) of cuprous chloride, were shaken mechanically until dissolved and tested in a capillary viscometer of the type described in the A.S.T.M. standard method (*1*). Two series were run, one with air and one with tank nitrogen, probably containing oxygen as an impurity.

The results in Figure 1 show that the deteriorative effect of atmospheric oxygen was eliminated by the use of 0.7 gram or more of cuprous chloride per 100 ml. of cuprammonium reagent, and that the degree of polymerization values approached those of tank nitrogen systems containing much less oxygen.

Values for the degree of polymerization were taken from a practically straight line relating log (time of flow) to degree of polymerization from DP = 300 to DP = 3000. Values for degree of polymerization were calculated using the expression derived by Battista (*2*),

$$\mathrm{DP} = 2160\ [\log(\text{relative viscosity} + 1) - 0.267]$$

Relative viscosity is the viscosity of the solution divided by that of the solvent (the viscosity of the solvents used in this work was 1.46 cp. at 20° C.). These viscosities were related to time of flow, *t*, as follows:

$$\text{Viscosity} = \text{density of solvent } (0.925 \text{ gram per ml. at } 20^\circ \text{ C.}) \times (0.000698\, t - 0.176/t)$$

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The two constants were obtained for the particular viscometer tube from time of flow measurements of two or more standard liquids of known viscosities obtainable at the National Bureau of Standards.

The upper curve also shows that the effect of the impurity of oxygen in the nitrogen, which must have been small in view of Scheller's results (*28*), was offset by small amounts of cuprous chloride. Similar experiments were performed without cuprous chloride, but (*a*) with copper metal and tank nitrogen, (*b*) with highly purified nitrogen giving no test for oxygen in a mass spectrometer, using special glass-stoppered flasks with inlet and outlet tubes to permit rigid exclusion of air, and (*c*) with special mixing vials completely filled with the cuprammonium reagent, to exclude air. All these measurements gave results essentially equal to those obtained using cuprous chloride.

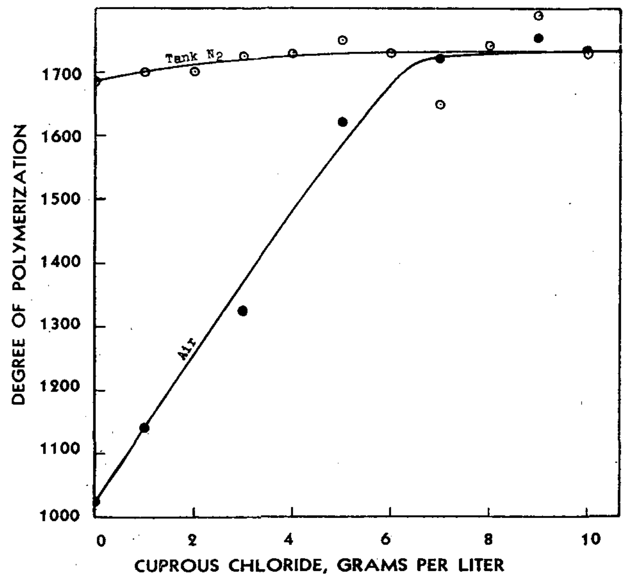

Figure 1. Effect of Cuprous Chloride on Depolymerization of Cellulose by Oxygen during Dispersion in Cuprammonium

There seems to have been no experimental investigation of the mechanism involved in these very simple methods of solving the problem of cellulose oxidation during dispersion.

Simple Preparation of Cuprammonium Reagent. The usual method of preparing the cuprammonium reagent by bubbling air through iced ammonium hydroxide containing copper turnings, as first proposed by Ost (*24*), is considered by some—for example, Hatch, Hammond, and McNair (*15*)—to be simple, but most investigations recorded in the literature do not appear to bear out this contention. The use of sucrose to facilitate dissolution of copper is almost always recommended. Experiences in three laboratories at the National Bureau of Standards indicate that the process is highly variable in facility of execution.

The method of preparing the cuprammonium reagent by oxidation of copper by air is complicated by the simultaneous oxidation of ammonia to nitrite ion. Nitrite ion, according to Clibbens and Geake (*6*), prevents the dispersion of higher grades of cellulose. Jolley (*18*) found that cuprammonium prepared by oxidizing copper metal with air, and containing nitrite (and sucrose), dispersed somewhat less cellulose than cuprammonium produced, without these contaminants, from copper hydroxide. This fact is reflected in all methods of this type by specifications for upper limits of the nitrite concentration (*1, 2, 6, 7, 15, 36*), which, if exceeded, call for discarding the batch.

These difficulties were recognized by Ost (*24*), who prepared cuprammonium reagent from basic copper sulfate, $Cu_2SO_4(OH)_2$, as well as by air oxidation of copper, but preferred the former because of its much greater simplicity. Others have prepared the reagent by dissolving copper hydroxide, $Cu(OH)_2$, in ammonium hydroxide (*9, 14*). The solubility of copper hydroxide in ammonium hydroxide is limited, corresponding to 11 to 12 grams per liter of copper. For this reason several investigators (*7, 31*) have objected to this procedure on the assumption that the resulting cuprammonium would be inferior for dispersing high-grade cellulose, and higher copper concentrations have been recommended, such as 30 (*7, 31*), 20 (*13*), and 15 grams per liter.

Evidence against the greater effectiveness of high concentration of copper has been presented by Hisey and Brandon (*16*), who showed that the time required to disperse a cotton cellulose in reagents containing 10, 15, 20, and 22.4 grams per liter of copper increased systematically over this range by a factor of 2. They ascribed this effect to a decrease in the surface area of the cotton by gel formation at higher copper concentrations. A similar opinion has been expressed by Joyner (*19*), who recommends 13 grams per liter of copper. Both Sakurada (*27*) and Kumichel (*20*) reported that all the types of cellulose except raw, untreated cotton were dispersible in cuprammonium containing 7 grams per liter of copper.

The solubility (11 to 12 grams per liter of copper) of copper hydroxide in ammonium hydroxide shows that cuprammonium, with a copper content (15 to 40 grams or more per liter) above its equilibrium concentration, as prepared by air oxidation of metallic copper, is unstable. The attainment of unstable systems is probably due to the partially colloidal condition of such cuprammonium, as shown by the work of Bhatnagar, Goyle, and Prasad (*3*) and Stamm (*33*). Such cuprammonium solutions can be expected to be less duplicable than those prepared from copper hydroxide.

The fact that the limited solubility of copper hydroxide is of

Table I. Degrees of Polymerization[a,b] and of Fluidity Values Obtained Using Cuprammonium[c] Reagents Prepared in Various Ways

Type of Cellulose	Copper Hydroxide		Oxidation of Copper		Basic Copper Carbonate	
	DP	Fluidity	DP	Fluidity	DP	Fluidity
Purified cotton[d]	2040	4.51	2110	4.15		
	2035	4.54	2075	4.33		
	2015	4.63	2100	4.21	Incompletely dispersed[e]	
	2015	4.62	2085	4.29		
	2050	4.46	2105	4.18		
Means	2031	4.55	2095	4.23		
Rag paper[f]	1795	5.98	1795	5.98	1875	5.43
	1795	5.96	1805	5.91	1885	5.39
	1795	5.96	1810	5.89	1870	5.47
	1785	6.05	1810	5.89	1850	5.61
	1775[g]	6.12	1795	5.98	1875	5.45
Means	1789	6.01	1803	5.93	1871	5.47
Sulfite paper[h]	590	27.7	590	27.7	590	27.7
	600	27.2	585	28.1	610	27.0
	590	27.7	590	27.7	610	27.0
	595	27.5	590	27.7	615	26.7
	600	27.5	590	27.7	610	27.0
Means	595	27.5	589	27.8	607	27.1

[a] See text for method of calculating DP from time of flow.

[b] Cellulose dispersions prepared according to method suggested in Procedure.

[c] Cuprammonium reagents contained 24 grams per liter of ammonia and 15 grams per liter of copper, except that prepared from copper hydroxide, which contained 11 to 12 grams per liter of copper. The reagent prepared by oxidizing copper metal with air also contained 1 gram per liter of sucrose and approximately 0.5 gram per liter of nitrite expressed as nitrous acid.

[d] Raw cotton purified by method of Corey and Gray (*8*).

[e] Undispersed masses of gel could be seen in purified cotton in cuprammonium made from basic copper carbonate. Periods of shaking were 16, 2, and 0.5 hour for purified cotton, rag paper, and sulfite pulp, respectively.

[f] Made in bureau paper mill from unused cotton fabric without bleaching or other serious degradative action. It probably represents highest type of cellulose encountered in machine-made paper. It was made without alum, sizing, or filler.

[g] DP values in Table I for rag paper cellulose are higher than those in Figure 1, which were obtained on paper stored for 8 years in a chemical laboratory, subject to higher room temperatures and infrequent and short daylight exposures. Values in table were obtained on paper stored in dark in a relatively cool place.

[h] Ordinary commercial sulfite pulp used in paper industry.

Table II. Degrees of Polymerization and Fluidity Values of a Cellulose[a] in Cuprammonium[b] Reagents Containing Varying Amounts of Nitrite, Sucrose, and Copper, and Prepared by Two Processes[c]

		DP	Fluidity
1.	Prepared from air and copper metal. Nitrite as HNO_2, 3.2 grams per liter; sucrose, 1 gram per liter; copper, 15 grams per liter	2535	2.58
		2500	2.68
		2505	2.67
		2535	2.58
		2515	2.64
	Means	2515	2.63
2.	Prepared from cupric hydroxide. Nitrite as HNO_2, 3.2 grams per liter; sucrose, 1 gram per liter; copper, 12.1 grams per liter	2460	2.81
		2420	2.92
		2420	2.94
		2425	2.92
		2470	2.77
	Means	244	2.87
3.	Prepared from cupric hydroxide. Nitrite as HNO_2, 3.2 grams per liter; sucrose, none; copper, 11.0 grams per liter	2405	3.03
		2455	2.82
		2515	2.64
		2465	2.80
		2440	2.88
	Means	2455	2.83
4.	Prepared from cupric hydroxide. Nitrite, none; sucrose, none; copper, 11. grams per liter	2425	2.91
		2410	2.96
		2475	2.77
		2490	2.71
	Means	2450	2.84

[a] Purified cellulose from different source, not to be compared with purified cellulose in Table I.
[b] All reagents contained 236 to 240 grams per liter of NH_3.
[c] Cellulose dispersions prepared according to Procedure.
For all other details see footnotes to Table I.

little consequence is due to virtual independence of degree of polymerization values on copper concentration in this range. This is shown most convincingly by the work of Hisey and Brandon (*16*), who found no significant difference in viscosity between cotton cellulose dispersions containing 10 and 15 grams per liter of copper; even those containing 22.4 grams per liter of copper were only slightly different. This was also shown by the work of Joyner (*19*), who found only slight differences at 29 and 10 grams per liter of copper, and by Neale and Waite (*23*). The writers confirmed these findings: using the rag paper cellulose described in Table I, found no differences in degree of polymerization in cuprammonium containing 10, 12, 14, 15, 16, 17, and 18 grams per liter of copper. The range of the data was of the same order of magnitude as the data in Table I. The experiments performed by the writers, and by others, all refer to dispersions of 0.5 gram of cellulose, the usual amount, per 100 ml. of cuprammonium. At higher concentrations of cellulose, the effect of copper concentration is appreciable.

The writers have found that all grades of cellulose studied are as effectively dispersed in cuprammonium prepared from cupric hydroxide as in that prepared by air oxidation of copper and having a higher copper concentration. There is a definite, but small, difference in the degree of polymerization and fluidity values for purified cotton with the two reagents as shown in Table I; for the other celluloses studied the differences are negligible. Application of the "Student's *t*" test (*32*) shows that this difference in behavior of purified cotton toward the two reagents is large enough to be significant. For most purposes, however, these differences are negligible.

In columns 6 and 7 of Table I are also shown some results obtained using a cuprammonium reagent prepared by dissolving basic copper carbonate, $Cu_2CO_3(OH)_2$, in ammonium hydroxide. This is similar to the reagent used by Ost (*24*); it does not disperse purified cotton completely.

The difference in degree of polymerization values for purified cotton in Table I was often confirmed in the course of this work, and found not to be due to differences in copper concentration. To ascertain whether it might be due to the nitrite present in the reagent prepared by the air oxidation of copper, experiments were performed in which ammonium nitrite was added to a cuprammonium solution prepared from copper hydroxide, and the results were compared with those using a cuprammonium solution containing an equivalent amount of nitrite by oxidation. Remembering that copper content in this range is unimportant, the results given in Table II indicate that no difference, either in degree of polymerization or dispersibility, was ascribable to nitrite or to sucrose. Colloidally dispersed copper, present in excess of the amount corresponding to the solubility of cupric hydroxide when cuprammonium is prepared by oxidizing metallic copper, appears to be the factor responsible for the difference in degree of polymerization values and fluidity which are obtained by the use of the two solutions.

It appears likely, therefore, that a cuprammonium reagent, prepared by simply stirring an excess of copper hydroxide with cold ammonium hydroxide, will adequately replace the reagent usually prepared in much more complicated procedures.

PROCEDURE

The cuprammonium reagent is prepared from concentrated, 29.0%, C.P. ammonium hydroxide, specific gravity 0.90, and C.P. copper hydroxide. The copper hydroxide is added to the ammonium hydroxide while stirring rapidly in an ice bath. [Copper hydroxide may be obtained from Eimer & Amend, New York, N. Y., or it may be prepared by Böttger's method given by Dawson (*9*) or Van Bemmelen (*38*). The writers found the commercial product to be equivalent to that prepared using Böttger's directions.] It is important that the ammonium hydroxide be cooled to at least 2° C. or below before adding the cupric hydroxide. To prevent undue loss of ammonia, the ammonium hydroxide solution should be chilled before it is poured from the reagent bottle. The escape of ammonia during mixing is diminished by stirring in a rubber-stoppered flask through a greased glass sleeve. After stirring about 15 minutes, the excess cupric hydroxide is allowed to settle. The concentration of ammonia is then usually upward of 240 grams per liter, at which point standardization may be desirable, as its concentration does affect the degree of polymerization values appreciably (*21*, *29*). Analysis of the reagent for copper is not necessary after the first few trials.

The cellulose dispersions are prepared by adding, in order, 0.231 gram (dry weight) of cellulose, corrected for moisture content, 10 to 20 1-cm. pieces of clean copper wire, 2- to 4-mm. diameter, about 0.4 gram of powdered C.P. cuprous chloride, and 50.0 ml. of the cuprammonium reagent, to a 125-Erlenmeyer flask. During the addition of these materials it is desirable, but not essential, to displace the air in the flask with tank nitrogen. The flasks are then rubber-stoppered and shaken, either by hand or mechanically, depending upon the quality of the cellulose, until dispersion is complete. (A commercial "wrist-action" shaker serves the purpose. Rubber stoppers offer a simple means of stoppering against the slight pressure of ammonia developed during the shaking, and were found to introduce no error.) The most convenient procedure is to leave the flasks on the shaker overnight and perform the viscosity measurement the next morning. Rich (*25*) and Howlett and Belward (*17*) have developed techniques which may prove advantageous when rapid dispersion is desired.

After dispersion, the viscosity may be measured by any one of a variety of techniques (*1*, *6*, *7*, *10*, *13*, *21*, *34*, *39*). The authors used a very simple device where the viscometer was set in a glass jacket through which water from a constant temperature bath was pumped continuously by a small centrifugal pump. The tip of the viscometer projected through a rubber stopper in the bottom of the jacket. A fluorescent lamp, with proper shading, behind the jacket made it easy to read the menisci.

The importance of good temperature control of the viscometer is shown by the fact that evaporation while pouring the solution into the viscometer can lower the temperature as much as 1° C. Therefore, it is best to allow the solution to stand in the viscometer about 3 minutes before starting the measurement.

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Determination of Safrole in Soap

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An analytical method based on the ultraviolet absorption characteristics of safrole in 9.5% aqueous alcohol has been developed for the determination of safrole in soap. The safrole is steam-distilled from the sample after treatment with silver nitrate to precipitate the soap. The distillate is examined in a Beckman DU spectrophotometer and the concentration of safrole is determined from the observed absorbence. Statistical evaluation indicates a reproducibility of results within the range ±6% of the safrole value when dealing with samples containing approximately 0.1% safrole.

AN ANALYTICAL method for the determination of small amounts of safrole (4-allyl-1,2-methylenedioxybenzene) in soap was needed for production control and to permit storage studies of its retention in soap. Previously developed gravimetric (*1, 3, 4*) and cryoscopic methods (*7*) were readily applicable only to large quantities of safrole. A method based on the ultraviolet absorption characteristics of safrole appeared to offer a possible solution. These absorption characteristics have been reported in the literature by several investigators (*2, 5, 6*).

A sample of Brazilian safrole of specific gravity (20/4) 1.096 and refractive index (20/D) 1.538 was used in this investigation. Because safrole is insoluble in water, some suitable solvent was necessary to enable study of its ultraviolet absorption characteristics. Absorbence–wave-length curves were determined with chloroform, 9.5% aqueous alcohol, and hexane as solvents. Reagent grade chloroform and hexane solutions of safrole gave satisfactory curves. When these organic solvents were saturated with water, the observed absorbence was greater than before saturation and consistent results could not be obtained. A 9.5% aqueous alcohol solution gave a satisfactory absorption curve in the Beckman spectrophotometer with absorption maxima at 285 and 232 mμ with slit widths of 0.80 and 1.6 mm., respectively. Because the 9.5% aqueous alcohol solution of safrole gave such satisfactory results, it was used as a standard solvent in the investigation.

To estimate safrole quantitatively, an accurate determination of its absorbence ($\log_{10}$ 100/per cent transmittance) was necessary to calculate a factor. Fifty observations were made to evaluate the safrole factor. Solutions of varying concentration from 1.096 to 4.384 mg. of safrole per 100 ml. in 9.5% alcohol-water were prepared and the absorbence at 285 mμ was determined in a Beckman Model DU spectrophotometer. Averages of the data are presented in Table I. The average that best suits the data is an absorbence of 0.207 per mg. of safrole per 100 ml. of 9.5% alcohol solution. The data show a satisfactory conformance to the Beer-Lambert law. Each independent observer should determine the absorbence for the safrole and the instrument used.

Separation of safrole from soap by distillation was attempted but only 25% of the oil was recovered. Recovery from 9.5% alcohol by steam distillation was then tried. Various amounts of safrole (2.0 to 9.0 mg.) were steam-distilled with water and 20 ml. of alcohol to make a 9.5% aqueous alcohol distillate. Two hundred milliliters of distillate were collected, followed by addition of 20 ml. of alcohol to the distilling flask and collection of

Table I. Determination of Safrole Factor in 9.5% Alcohol Solution

Safrole, Mg./100 Ml.	No. of Observations	Av. Absorbence at 285 mμ, $\log_{10}$ 100/% Trans.	Av. Safrole Factor, Absorbence/ Mg./100 Ml.
1.096	10	0.226	0.207
1.534	10	0.318	0.208
2.192	10	0.453	0.207
3.288	10	0.678	0.206
4.384	10	0.897	0.205

Table II. Loss of Safrole in 9.5% Aqueous Alcohol after Refluxing with Various Reagents

(Calculated from absorbence of flask contents)

Reagents in Reflux Flask	Loss of Safrole: 15-minute reflux	Loss of Safrole: 45-minute reflux
	%	%
Distilled water	8.3	42.5
0.5 *N* acetic acid	5.6	27.4
0.5 *N* sulfuric acid	6.6	...
0.5 *N* sodium hydroxide	7.3	40.3

200 ml. more of distillate. An average recovery of 83.8% of the safrole initially present was obtained in the first portion. No appreciable absorbence at 285 mμ was found either in the second portion of distillate or in the residue in the distilling flask.

The effect of the presence of acid and alkali in the distilling flask on the recovery of safrole was studied by refluxing 9.5% aqueous alcohol solutions of safrole with several reagents for periods of 15 and 45 minutes. The values obtained by measurement of the absorbence of the flask contents, as shown in Table II, show loss of safrole to be least in the presence of a weak acid. In confirmation of this, steam distillation of a 9.5% aqueous alcohol solution of safrole containing 0.5 *N* acetic acid yielded an average safrole loss of 5.5% for five determinations.

Inasmuch as the best recovery of safrole after refluxing was obtained in the presence of acetic acid, safrole was steam-distilled from a soap solution which had been acidified with acetic acid. This proved ineffective because the distillates were very turbid, interfering with the observation of their ultraviolet absorption characteristics. Safrole is retained by filter paper; thus filtration could not be used to clarify these turbid distillates. Various precipitants for soap were tried with the acid, but the distillates were still turbid. A steam trap inserted between the distilling flask and the condenser failed to eliminate the difficulty. Apparently, fatty acid constituents of the soap were distilling over. The use of acid was discontinued to prevent the formation of a fatty acid layer in the distilling flask.

Table III. Soap Turbidity Correction Terms for 9.5% Aqueous Alcohol

Weight of Soap, Grams	No. of Determinations	Term, $\bar{x}$ (Absorbence)	Standard Deviation
1	8	0.024	0.006
3	8	0.056	0.007
5	8	0.078	0.010
10	8	0.118	0.015

Standard deviation = $\sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$.

where $\bar{x}$ = average
x = individual observations
n = number of observations

A study of many metallic salts as soap precipitants was made. Silver nitrate was found to be the best reagent because the silver salt formed was the only insoluble soap that did not coalesce to a semifluid mass on prolonged heating. The maintenance of a granular structure by the silver soap was considered desirable because it lessened the observed tendency toward occlusion of safrole. The excess silver nitrate apparently provides the necessary conditions to prevent the loss of absorbence indicated in Table II. The steam distillates from mixtures of safrole, soap, and silver nitrate solutions showed a very slight turbidity which could not be dispelled by increasing the alcohol concentration to as high as 50%.

Correction terms for the observed absorbence to eliminate the error due to this turbidity were determined. Varying amounts of soap, water, silver nitrate solution, and alcohol to make 9.5% alcohol in 200 ml. were steam-distilled. A known amount of safrole was added to the distillates and the absorbence was observed. The soap correction term was calculated by subtracting the absorbence due to the known safrole from the observed absorbence. The correction term was not determined from the direct reading of the turbid distillates from a reagent blank because of the error inherent in the extremes of the absorbence scale of the spectrophotometer. The correction terms obtained, shown with their standard deviations in Table III, are for the soap used in this investigation and may be expected to vary with different soap types.

A series of recovery tests from steam distillation was made using 1 gram of soap, 5 ml. of 20% silver nitrate solution, varying quantities of safrole ranging from 1.0 to 4.5 mg. per 100 ml., and alcohol to make a 9.5% solution in 200 ml., the volume of distillate collected. An average recovery of 95.5% of the safrole was obtained in twenty determinations. These results are corrected using a soap turbidity term as described above. Average recoveries with different quantities of safrole originally present are shown in Table IV. Standard deviations are also given as a measure of the dispersion of the data.

Table IV. Recovery of Safrole by Steam Distillation from Solution of Safrole, Silver Nitrate, and 1 Gram of Soap

Original Concn. of Safrole, Mg./100 Ml.	No. of Detns.	Safrole Recovered, Mg./100 Ml.	Recovery, %	Standard Deviation
1.096	4	1.059	96.6	4.0
1.534	4	1.473	96.0	4.3
2.192	4	2.085	95.1	3.4
3.288	4	3.127	95.1	1.1
4.384	4	4.160	94.9	1.3

Average recovery (20 observations) = 95.5%
Standard deviation (20 observations) = 2.9

ANALYTICAL METHOD

Apparatus. Standard all-glass steam-distillation equipment with 2-liter steam generator and 1-liter distillation flask. A steam trap is used between the distilling flask and the condenser, and an adapter feeds the condensate to the receiver.

Reagents. Silver nitrate solution, 20% in water. Ethyl alcohol, 95%.

Procedure. Weigh a quantity of sample estimated to contain about 4 mg. of safrole and transfer into the distilling flask. Then introduce 20 ml. of ethyl alcohol and enough water to dissolve the soap. Add silver nitrate solution (5 ml. per gram of soap are usually sufficient) with constant swirling to precipitate the soap. Connect the steam trap and condenser and attach an adapter leading into a wide-necked 200-ml. volumetric flask. Add water to the boiling flask, heat, and connect to the distilling flask. Control the rate of boiling to complete distillation in about 20 minutes. Collect exactly 200 ml. of distillate in the volumetric flask, mix well, and measure its absorbance in the Model DU Beckman spectrophotometer at 285 mμ.

Calculations.

$$\frac{\text{Observed absorbence} - (\text{soap turbidity term for wt. of soap used})}{216} = \text{mg. of safrole/100 ml. of distillate}$$

$$\frac{\text{Mg. of safrole per 100 ml. of distillate} \times 0.1}{\text{Grams of sample wt. equivalent to 100 ml. of distillate}} = \%\ \text{safrole in original sample}$$

The figure 216 was calculated from the experimentally determined factor on the basis of the observed recovery of safrole by steam distillation from soap.

Time required per analysis, 45 minutes.

APPLICABILITY OF METHOD

The method described has been used in this laboratory for about 6 months and has been found entirely reliable. Using this procedure, it has been possible to determine as little as 0.004% safrole in soap. During the investigation, it became necessary to determine safrole in soap containing other known perfume constituents. In this specific case, it was found possible to perform the analysis by changing the solvent to 50% aqueous alcohol and by redetermining the necessary factor and terms.

In addition to soap analysis, the method has been applied to the determination of safrole where present in known detergent and/or water-softening formulations. In these cases, 1 gram of soap and 5 ml. of 20% silver nitrate solution were added to permit using the recovery data already developed for soap. This would have been unnecessary if recoveries had been developed for the specific formulations being analyzed.

The method is not general for the determination of safrole because of possible interference from other constituents in unknown safrole-containing perfumes. However, the method can be used directly when safrole is the only perfume constituent in soap. The method can be applied to the analysis of safrole in soaps with known perfume formulations where the interference from other constituents can be determined and the necessary correction made.

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Determination of 2,4-Dichlorophenoxyacetic Acid in Soil

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A method for determining 2,4-dichlorophenoxyacetic acid in soil consists of leaching the soil with water, acidifying and extracting the leachate with ethyl ether, evaporating the extract to dryness, and partitioning the residue between tributyl phosphate and phosphate buffer of pH 7. For the distribution between the two solvents, a 24-plate countercurrent distribution procedure, using the Craig instrument, was performed. The concentration of 2,4-D in the tributyl phosphate layer of each tube was determined by measuring the extinction at a wave length of 284 mμ. From the amount found in a selected series of tubes the concentration of 2,4-D in the original sample can be calculated.

IN SPITE of the extensive use of 2,4-dichlorophenoxyacetic acid as a herbicide and a growth-regulating substance, no satisfactory chemical method for its determination in complex mixtures has been described. Such a method would be particularly valuable in studies involving the retention and movement of 2,4-D in various types of soils, and also, if sufficiently sensitive, for investigating the uptake of 2,4-D by plants.

Studies of this type have been reported by Hanks (*3*), who used a modification of the bioassay method of Swanson (*6*). This method involves the inhibitory effect of 2,4-D on the elongation of the primary root of corn seedlings. It was shown that a relationship exists between the concentration of 2,4-D and the root length after 48 hours' growth. Although the bioassay method is extremely sensitive—working best in the range of 0.01 to 2.0 p.p.m.—it is not sufficiently specific for 2,4-D; any substance that inhibits the growth of the root will be included in the estimate for 2,4-D and, therefore, the method may yield erroneously high results. Bandurski (*1*) determined 2,4-D in soil leachates spectrophotometrically by measuring the extinction at a wave length of maximum absorption—i.e., 284 mμ. The relationship between concentration and absorption was found to obey Beer's law within 5%. Good results were reported on soil leachates, providing no other interfering substances were present. It was recommended that, for low concentrations of 2,4-D, the acidified leachate first be extracted with several portions of ethyl ether and the extinction compared with a blank which had been treated in the same manner. Because soil leachates usually contain substances that absorb light of a wave length of 284 mμ, the procedure is not specific for 2,4-D.

A very sensitive colorimetric method for detecting 2,4-D has been described by Freed (*2*). When 2,4-D is heated at 150° C. for 2 minutes with a solution of chromotropic acid in concentrated sulfuric acid, a red color is produced. As little as 0.05 microgram per ml. of 2,4-D can be detected. The method, however, has not been satisfactory for quantitative estimations. In addition, a number of other organic compounds have been found to produce a color that interferes with the determination. Rooney (*4*) described a procedure for the determination of 2,4-D and its compounds in commercial herbicides. Essentially, the method consists of the determination of 2,4-D by first extracting it from any inert material with ethyl ether and then titrating the acid group. A check is made also by determining the total chloride content. It is apparent that acids and halogens will affect the reliability.

The method described in this paper for the determination of 2,4-D in soils combines the principle of countercurrent distribution with the spectrophotometric procedure of Bandurski.

The technique of applying countercurrent distribution methods to the analysis of complex mixtures of chemically similar substances has been reported by several groups of workers (*5, 7–9*). In this study the practice outlined in a previous publication by the present authors on the determination of phenol and *m*-cresol in mixtures (*8*) was followed. Specifically, the steps entailed in the determinations are: The soil is leached with a relatively large quantity of water; the leachate is acidified with strong sulfuric acid and extracted twice with ethyl ether; the ether extract is evaporated to dryness and the residue dissolved in a definite volume of phosphate buffer of pH 7; an aliquot of this solution which contains the dissolved 2,4-D is subjected to a 24-plate countercurrent distribution between phosphate buffer of pH 7 and tributyl phosphate; a sample of the tributyl phosphate layer from each tube is examined spectrophotometrically at a wave length of 284 mμ; and from the extinction measured, calculations can be made to determine the concentration of 2,4-D in the leachate and consequently in the soil itself.

DISCUSSION

Because there are present in soil leachates substances which absorb light in the same wave length range as 2,4-D, a direct spectrophotometric method for determining this compound was not feasible. In view of the successful use of countercurrent distribution techniques for separating chemically similar substances, an application of this principle was considered in the present problem. Various solvents were tested to find a system with a partition coefficient for 2,4-D sufficiently different from the interfering substances so that a satisfactory separation could be achieved. Carbon tetrachloride, chloroform, benzene, and butanol were used in combination with various buffers over a wide range of pH values. None of these systems was favorable for partitioning the 2,4-D. It was found eventually that with the system tributyl phosphate–phosphate buffer, pH 7, 2,4-D had a partition coefficient of approximately 1. Variation of the pH of the buffer changed the partition coefficient; consequently, a large volume of phosphate buffer with a large buffer capacity (0.5 *M*) was prepared and used throughout the experiments. As it was found that the tributyl phosphate remained cloudy upon shaking with the buffer, a preliminary treatment was considered advisable. When this cloudy solution was filtered and reshaken with the phosphate buffer, the apparent suspended impurities were no longer visible and a clear solvent, suitable for use in the Craig apparatus, resulted.

Of particular interest is the fact that the procedure described can be used for the determination of a wide range of concentrations of 2,4-D in the soil. For low concentrations, a very large soil sample can be leached, for this is ultimately reduced to a small volume by extracting the leachate with ethyl ether and evaporating to dryness before making up to the final volume; for large concentrations the final dilution can be made proportionately larger.

Attempts to determine 2,4-D by simply extracting the acidified soil leachates with various solvents and measuring the extinction proved unsatisfactory, because the interfering substances were also partially extracted.

It was possible to effect a distinct separation of 2,4-D from the other products present in soil leachates by means of the countercurrent distribution method.

For the final determination of the concentration of 2,4-D in each of the tubes, the ultraviolet absorption measurements appeared the simplest. It was found that the absorption spectrum was the same in water and tributyl phosphate, and that the extinction in the organic solvent was also directly proportional to the concentration. Consequently, the concentrations of the supernatant tributyl phosphate layers only were determined and used as an indication of the distribution of 2,4-D throughout the Craig apparatus.

APPARATUS AND REAGENTS

The standard solution of 2,4-D that was used contained 1 mg. per ml. and was prepared by weighing out the pure material (melting point 140–141° C.) and diluting to the proper volume.

The two immiscible solvents used for the countercurrent distribution were tributyl phosphate and 0.5 *M* phosphate buffer at pH 7.0.

The Craig apparatus was a 24-tube stainless steel unit which had a capacity of 8 ml. for each tube of the lower section.

The extinction measurements were made with a Beckman quartz spectrophotometer, Model DU, using 1.0-cm. quartz cells.

PROCEDURE

Leaching of Soil. Because it was particularly desirable to be able to determine very small concentrations of 2,4-D in the soil, a sufficiently large soil sample was selected so that the aqueous leachate would contain at least 1 mg. of 2,4-D. In the present study 2 pounds of dry soil were treated with a total of 1 mg. of 2,4-D dissolved in 40 ml. of water, and allowed to stand for 4 hours. The treated soil was then leached with 1500 ml. of water by allowing 50-ml. portions to percolate slowly through the sample contained in a glazed earthen crock 4 inches (10 cm.) in diameter which was provided with a drain near the bottom.

The entire leachate was transferred to a 2-liter separatory funnel, acidified with 100 ml. of 1 to 1 sulfuric acid, and extracted twice with ethyl ether (first with 200 ml. and then with 100 ml.). The ether extracts were combined in a suction flask and the solution was evaporated to dryness by aspirating with a water pump. The dry residue, which contained the 2,4-D as well as other interfering compounds present in the soil, was dissolved in 10 ml. of the phosphate buffer (previously saturated with tributyl phosphate). An 8-ml. aliquot of this solution was used for the countercurrent separation.

COUNTERCURRENT DISTRIBUTION. The details of the technique for the operation of the Craig machine have been described elsewhere, and are not repeated here. The instrument is filled with the proper quantity of the two immiscible solvents, and the sample, introduced into the zero tube, is submitted to a 24-plate countercurrent distribution between the two solvents. Because tributyl phosphate is less dense than the phosphate buffer, it was found convenient merely to determine the extinction of the upper layers.

SPECTROPHOTOMETRIC DETERMINATION. An aliquot of the upper tributyl phosphate layer of each tube was carefully removed and introduced into the quartz cell. The extinction was measured at a wave length of 284 mμ against a sample of pure tributyl phosphate which was saturated with the buffer.

CALCULATIONS

A stepwise description of a sample calculation has been presented (*8*). The identical procedure was followed in this study.

By inserting the proper values in the formula

$$T_{n,r} = \left(\frac{n!}{r!(n-r)!}\right)\left(\frac{1}{k+1}\right)^{n}(k)^{r} \tag{1}$$

the fraction of the original concentration of 2,4-D in tube *r* can be readily calculated. In the above expression $T_{n,r}$ = fraction of original substance in tube *r*, *n* = total number of transfers, and *k* = partition coefficient. The partition coefficient, *k*, is determined from the formula

$$k = \frac{1}{F} \times \frac{T_r}{T_{r-1}} \tag{2}$$

where *F* is the factor for relating T_r to T_{r-1}, and T_r and T_{r-1} are the amounts of solute in tubes *r* and *r* − 1. *F* is in turn calculated by the formula,

$$F = \frac{n+1-r}{r}$$

In the calculations described in the preceding paragraph, the partition coefficient, *K*, is calculated from Equation 2, using the extinction values of the tributyl phosphate layers of tubes 11, 12, 13, and 14 following a 24-plate extraction; the average of these four values was used in Formula 1. Because the extinction is directly proportional to the concentration of 2,4-D, the amount present in the tributyl phosphate layers can be determined by comparing the extinction obtained to that found for a standard solution at a wave length of 284 mμ. For the determination in an unknown sample, it is necessary to assay only about 4 tubes; those near the peak of the distribution curve are most suitable.

Inasmuch as the value $T_{n,r}$, obtained in the expression above, is merely a fraction of the original amount, it is divided into the 2,4-D content found in the tributyl phosphate layer of tube *r* to give the sum of the 2,4-D present in all the tributyl phosphate layers. In order to determine the quantity of 2,4-D left in the buffer layers, this latter value is multiplied by the partition coefficient. The sum of both layers is the amount of 2,4-D in the original sample.

After the quantity of 2,4-D in the aliquot sample subjected to the partition distribution has been determined, it is a simple matter to calculate the concentration in the original sample by multiplying by the dilution factor.

Table I. Recovery of 2,4-D from Aqueous Solutions by Ether Extraction

Sample No.	Concentration of 2,4-D: Original solution	Concentration of 2,4-D: Solution from ether extracts	Error
	γ/ml.	*γ/ml.*	%
1	7.8	8.0	+2.6
2	22.1	21.8	−1.4
3	24.5	24.6	+0.3
4	28.5	28.2	−1.0
5	57.0	57.2	+0.5
		Av.	±1.2

RESULTS

The efficiency of several steps in the procedure, such as the ether extraction and distribution between buffer and tributyl phosphate, was investigated. When an acidified aqueous solution of 2,4-D was extracted with several portions of ether, all the 2,4-D was found to be present in the ether layer. This was shown by (1) measuring the extinction of the solution before extraction, and (2) after extracting, evaporating the ether, and dissolving the residue in the same volume of water as the original solution. The accuracy of the recovery for several concentrations of 2,4-D is shown in Table I.

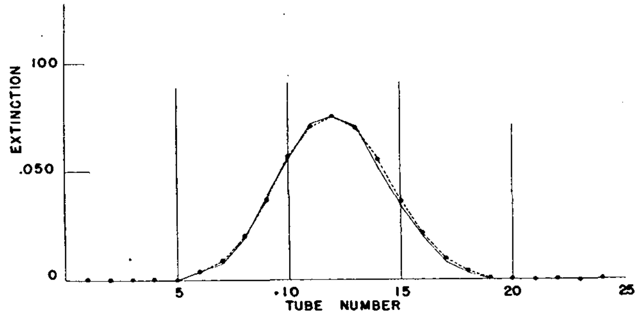

Figure 1. 24-Plate Countercurrent Distribution of 2,4-D

Dotted line. Experimental values for distribution of 800 γ
Solid line. Calculated theoretical values

The successful use of the Craig apparatus for separating 2,4-D from other substances in soil which interfere with the spectrophotometric determination is summarized in Figures 1 and 2. Figure 1 shows a 24-plate distribution of pure 2,4-D and the theoretical distribution as calculated from the binomial expansion theorem. Curve 1, Figure 2, was obtained from a soil sample which was pretreated with 1 mg. of 2,4-D and analyzed by the procedure described; curve 2 shows the results with untreated soil. The quantitativeness of the method is illustrated in Table II.

Table II. Determination of 2,4-D in Soil

Sample No.	Amount Added	Amount Found	Error
	Mg.	*Mg.*	%
1	20.0	19.2	−4.0
2	15.0	14.4	−4.0
3	4.00	4.20	+5.0
4	4.00	3.91	−2.5
5	1.00	0.98	−2.0
6'	1.00	1.06	+6.0
7	1.00	1.00	0.0
8	1.00	0.95	−5.0
		Av.	±3.6

The Craig machine was also successfully used for separating 2,4-D from 2,4-dichlorophenol, the most likely contaminant of synthetic 2,4-D. This was established by submitting a mixture containing 4 mg. each of 2,4-D and 2,4-dichlorophenol to a 24-plate countercurrent extraction, and then determining the extinction of the tributyl phosphate layers. Two distinct peaks were obtained with maxima in tubes 3 and 12. Because the position of the second peak corresponds to that obtained for 2,4-D alone, it is concluded that the peak with its maximum in tube 3 is due to 2,4-dichlorophenol. From this study it is apparent that any 2,4-dichlorophenol present in a synthetic sample of 2,4-D will not interfere with the determination in soil by the present method.

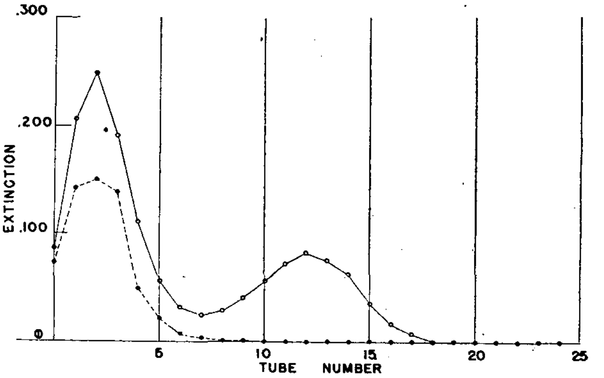

Figure 2. 24-Plate Countercurrent Distribution of Extracted Soil Leachates

Solid line. 1500-ml. leachate from soil treated with 1 mg. of 2,4-D in 2 pounds
Dotted line. 1000-ml. leachate from untreated soil

An early experiment, in which the soil was pretreated with 40 mg. of 2,4-D and leached with successive 500-ml. portions of water, showed that the first leachate contained approximately 20 mg. and the second leachate contained 16 mg. of 2,4-D; no additional leaching was performed. Because of the low solubility of 2,4-D in water it is advisable to continue leaching the soil with water until the leachates no longer contain any 2,4-D. In the present studies, Frankstown loam was the soil used. As it was found that leaching 2 pounds with 1500 ml. of water gave quantitative recoveries of added 2,4-D, no attempt was made to establish the minimum amount of leaching required. Undoubtedly, the volume of water necessary to leach 2,4-D completely from various types of soil will depend on the nature of the particular soil involved. This, of course, should be determined experimentally with each variety of soil under investigation.

Table III. Chemical and Biological Determination of 2,4-D in Soil

Length of Contact	Originally Present	Amount Found: Chemically	Amount Found: Biologically	Average Recovery: Chemically	Average Recovery: Biologically
Days	*Mg.*	*Mg.*	*Mg.*	%	%
0	4.00	3.91	3.85, 5.00	97.5	110
14	4.00	0.77	0.75, 0.78, 0.90	19.2	20.2

As a final experiment on the specificity of the method, it was desirable to ascertain whether the results obtained by the procedure described are in agreement with the values obtained by the biological method. For this purpose, a sample of soil was treated with 2,4-D and the amount initially present was determined by both methods; a similar determination was made after the 2,4-D was permitted to remain in contact with the soil for 2 weeks. From the results shown in Table III it appears that there is a loss of over 80% of the 2,4-D after an interval of 2 weeks. This loss is demonstrated both chemically and in physiological activity. Inasmuch as the values obtained in both methods parallel each other (and are in close agreement), it is concluded that the procedure herein described is actually determining the compound possessing the physiological activity characterized by 2,4-D.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Paul K. Brown for his valuable assistance in these experiments. Thanks are also extended to H. Robert DeRose for his cooperation in performing the leaching of the soil samples and to Robert Weintraub for determining 2,4-D on several samples by the biological method.

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Instrument for Controlled-Potential Electrolysis

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An instrument is described which will usually hold the cathode-solution potential, measured against a reference half-cell, to ±2 mv. over a 24-hour period. Oscillograms show that large rapid periodic fluctuations in cell potential, which are occasionally encountered in the deposition of trace amounts of an element, may strikingly broaden the limits of control. The effects of changes in the electrolyte, the electrode material, and the impressed voltage on the fluctuations were examined briefly.

ELECTRODEPOSITION is a common method for separating and determining many elements present in solution. Because an electrolysis involves only a few simple operations and can be easily carried out by remote control, the method is a very promising one for the separation of radioactive elements.

Some of the existing electrolytic procedures could be improved and many new procedures introduced by carefully selecting and regulating the cathode-solution potential. Sand (*12*) and his coworkers were the first investigators to carry out successful experiments. Using various metals in combination with different solutions of electrolytes, Sand obtained potentials from dissolution of the metals which he impressed upon a second "inert" electrode. Although the potentials were fairly constant for long periods of time, it was often difficult to obtain the desired value without resorting to a somewhat cumbersome experimental setup. The difficulty was finally eliminated by the development of instruments for the regulation of cathode-solution potentials.

INSTRUMENTAL ASPECTS

Varying degrees of automatic control of cathode-solution potential have been attained by previous workers. In 1942 Hickling (*6*) reported on a regulator which he designed, using all-electronic control. Anode current was passed from a battery through a triode which had its grid potential controlled by the cathode-solution voltage variation from a fixed reference, through a thyratron and an intermediate vacuum tube. Although electronic in nature, the circuit was slow, taking 1 to 5 seconds to respond to an out-of-balance condition. It would control in both directions, but had poor sensitivity and was subject to drift, resulting from changes in the thyratron control characteristic.

Over the past few years, Diehl (*1*) and his associates have developed a regulator which gives closer (ca. ±10 mv.) regulation, but compensates for changes only in one direction. Plating current up to 10 amperes and 20 volts is supplied from a full-wave selenium rectifier. The rectifier is fed from a Variac which is turned by an amplifier-controlled motor to decrease voltage in order to limit the plating current and thereby prevent the cathode-solution potential from exceeding a preset value. Cathode-solution potential changes are amplified by a direct current amplifier and used to actuate a relay in the motor circuit.

Lingane (*9*) has pointed out that in many cases control in both directions is essential, and he has built and used a very simple regulator that will do this. Plating current is supplied to the cell through a motor-driven rheostat. The motor does not turn when the solution potential is correct, but turns when the potential differs from the preset value by an amount sufficient to actuate the relay. Cathode-solution potential is in series opposition with the preset voltage on the slide wire. Differences cause current to flow in the circuit, which also contains a contact-making galvanometer that controls motor rotation. With the motor running freely, the potentiometer makes a full turn in 5 minutes. Sensitivity is about ±20 mv. with negligible drift; the probe current is several microamperes.

Heyd, Ohmart, and Hites (*5*) have developed an electronic regulator which has many good features. It is electronic in nature, having fast response. It controls in both directions, and to within 1 mv. over a short time. However, over 24 hours a drift of several millivolts may be expected. This regulator passes all current through vacuum tubes, and is designed to supply up to 60 ma. Its chief limitation is low current capacity; a comparatively minor disadvantage is the possibility of a small "zero" drift during a prolonged electrolysis.

More recently, an instrument using rectified alternating current has been described (*10*), as well as one that can perform other related tasks (*8*).

The present instrument employs a mechanical regulation of the potential, which is faster and permits closer control than earlier regulators of this type. Although electronic regulation may be preferable because of the instantaneous response, its extension to current ranges of 1 ampere and more is more cumbersome.

DESCRIPTION OF INSTRUMENT

Figure 1 is a photograph of the instrument, and Figure 2 is a schematic wiring diagram.

The plating current is obtained from storage cells and will supply up to 1 ampere and 18 volts. Control is within ±2 mv.

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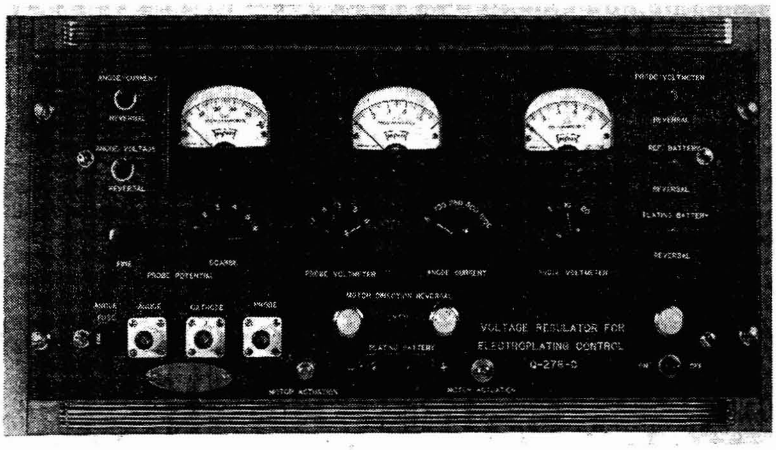

Figure 1. Instrument for Controlled-Potential Electrolysis

for slow changes, and the regulator works equally well in either direction. "Regulation," defined as absolute departures from a voltage setting, may be greater than 2 mv. in exceptional cases where extremely rapid changes in potential are encountered. Therefore, if periodic cathode phenomena occur, no definite figures can be given for the limits of regulation, but a study of the oscillograms in Figure 4, especially *B* and *C*, shows the regulator to be beneficial, though too slow in response to compensate completely for such changes.

The desired cathode-solution voltage is set on the reference slide wire, using a potentiometer connected between the probe and the cathode. After the desired potential is set up on the slide wire attached to the reference battery in such a direction as to oppose the cathode-solution plus calomel half-cell potential, changes in the cathode-solution potential cause a current to flow through the converter (Part No. 75829-1, Brown Instrument Company, Philadelphia, Pa.) which changes the direct current into 60-cycle alternating current. Because the converter is driven at 60 cycles by a coil connected to line frequency, the phase of this inverted direct current with respect to that of the line depends only on the direction of flow of the direct current. Reversal of the "probe" signal current causes a 180° phase reversal in the alternating current which leaves the converter and flows through the input coupling transformer. This is amplified in the 4-stage alternating current amplifier (Part 76020-1, Brown Instrument Company, except for the higher impedance input transformer and the inverse feedback between the output and second grid) and fed to one phase of the two-phase motor (Part 76750-3, Brown Instrument Company). The other phase is connected to the line. Therefore, the motor will remain fixed if there is no signal, or run in a direction determined by the direction of flow of direct current in the probe circuit.

The motor contains some velocity damping due to the zero-signal direct current (about 16 ma.) flowing through the two 7N7 output tubes. Further damping current, up to 44 ma., is supplied by the small selenium rectifier circuit. This heavy velocity damping prevents overshooting and instability under unusual conditions which are discussed more fully below. The instrument anticipates its balance point because of capacitive coupling between anode and probe, and so it slows down as the balance point is approached. This further discourages hunting, allowing the instrument to be used with full gain under all conditions. At full gain, the motor will rotate for a 2-mv. unbalance, and cathode potential will be regulated to ±2 mv. If it is desired to use the device as a simple two-terminal regulator, the probe and anode leads may be connected together. Under these conditions a constant potential will be maintained between anode and cathode.

An interesting point in connection with the General Radio, Type 200B Variac used as a direct current potentiometer is the smoothing effect of the carbon brush. This Variac has about 500 turns, which results in 24 mv. per turn when used with a 12-volt plating battery; yet the regulator will maintain anode-cathode voltage within 5 mv., indicating that the brush, which contacts several turns at once, smooths out to a large extent the potential steps between turns.

Meter shunts are not given in Figure 2. Convenient current ranges are 5, 100, 250, 500, and 1000 ma.; anode voltage ranges, 1, 10, and 20 volts; and probe reference voltages, 0.1, 0.5, 1.0, and 5.0 volts. The probe voltmeter requires 50 μa. for full-scale deflection.

Referring to Figure 2, S_1 and S_2 are limit switches which stop the motor if the Variac brush reaches either end of the winding. If this happens, L_1 or L_2 will light. After proper adjustments have been made, the regulator may be started again by pressing the corresponding push button.

In some cases it is desired to change the cathode-solution potential to a new setting without interrupting the electrolysis. If reversal of sign (*vs.* the reference cell) is anticipated, the situation can be readily handled by connecting on additional battery in series with the cell anode and the regulator.

The 4000-μfd., 25-volt electrolytic condenser shown between anode and cathode is for the purpose of reducing transients caused by variations in brush contact resistance. This is not noticeable at low current, but at high current the condenser is required.

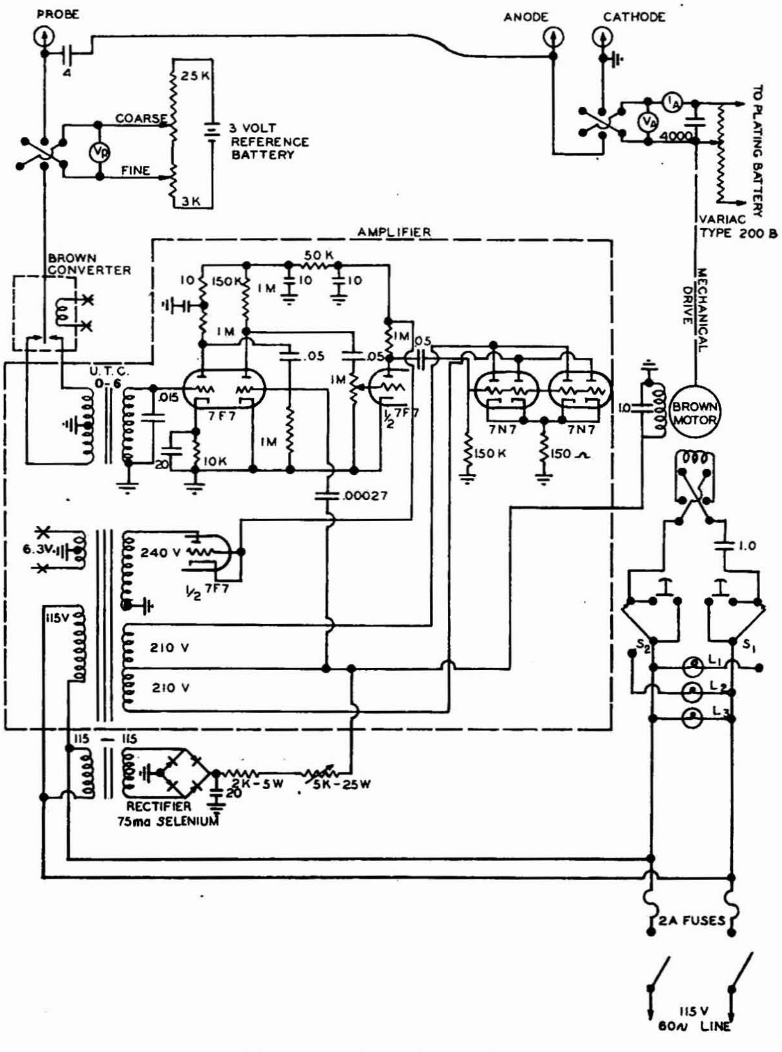

Figure 2. Regulator Circuit

Table I. Experimental Detail for Oscillograms

No.	Solution	Cathode	Stirring	V_{CP}[a]	V_{AC}[a]	I_A[a]	Total Swing, Mv.	Remarks
3A	0.1 *M* NaCN 1.0 *M* + NaOH (10^{-3} *M* Ag^+)	Pt	Yes	−1.300	4.4	1.1	1.5	Regulator on, good control
3B	0.1 *M* KCN	Pt	Yes	−0.760	3.5	0.40	17	Regulator on, poor control
3C	0.1 *M* KCN	Pt	Yes; No	−0.760	3.5	0.4, 0.1	28	Stirrer started and stopped
4A	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	−0.5000	2.2	0.102	3	Beginning of variations
4B	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	−0.700	2.6	0.20	7	Regulator on, approximately 1.75 cp., irregular
4C	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	−0.700	2.6	0.20	15	Approximately 1 cp., irregular
4D	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	−0.900	2.6	0.23	40	Spasmodic 1-cycle variations, 3 to 7 sec. apart
4E	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	−1.000	3.1	0.35	180	Spasmodic 1-cycle variations, 22 sec. apart
4F	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	−1.100	3.3	0.38	320	Spasmodic variations about 63 sec. apart
4G	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	−1.200	3.5	0.45	5	Approximately 1 cp.
5A	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	+0.500	−2.2	−0.30	9	About 0.5 cp.
5B	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	+0.700	−2.6	−0.40	5	About 0.75 cp.
5C	0.1 *M* NaCN plus 1.0 *M* NaOH	Pt	Yes	+0.800	−3.0	−0.70	2	Beginning of appreciable conduction
6A	1.0 *M* $HClO_4$	Ta	No	+5.000	−6.9	−0.50	95	Predominant sawtooth at about 300 cp., pattern continually changing, many frequencies up to 1 kc. on CRO
6B	1.0 *M* $HClO_4$	Ta	No	+6.000	−8.0	−0.60	35	Regular pulses, 1 cp.
6C	1.0 *M* $HClO_4$	Ta	No	+4.000	−5.3	−0.10	16	Regular pulses, 1 cp.
6D	1.0 *M* $HClO_4$	Ta	No	+3.000	−3.9	0.00	3	Regular pulses, 1 each 5 seconds
6E	1.0 *M* $HClO_4$	Ta	Yes	−1.100	+2.9	0.50	3	Regular pulses, 1 each 5 seconds
7	Concd. $CuSO_4$ + 0.3 *M* H_2SO_4	Pt	Yes	−1.100	+8.2	660	44	Variation highly erratic, internal anode of Pt

[a] V_{AC} = anode-cathode potential, volts.
V_{CP} = cathode-probe potential, volts.
I_A = current flowing into solution from anode, ma.
[b] Regulator off in all cases except as noted.

Because a direct current amplifier is not used, the only drift is that due to the reference batteries, and this has been found ordinarily to be less than 1 mv. in 24 hours. The instrument responds to any unbalance over 2 mv., with a speed such that one full turn of the current-regulating Variac takes about 10 seconds. Probe current is negligible, because 0.1 μa. is sufficient to initiate corrective action. Because the instrument amplifies alternating current only, it may be turned on and used immediately without any long warmup period.

TESTS OF REGULATOR PERFORMANCE

Experimental Setup. Tests were made with various chemical solutions to determine how closely the regulator could maintain the cathode-solution potential, using an electrolysis setup described elsewhere (*2*) and 12 volts across the Variac-potentiometer.

The cell consisted of a piece of 25-mm. inside diameter glass tubing about 8 cm. long, pressed down onto a sheet of platinum which served as the cathode. The resulting cathode surface area exposed to the solution was 5 sq. cm. The thin metal cathode was backed up by a thick piece of smooth brass, with a sheet of insulation between and the glass tube held down tightly by means of a clamp. The solution to be electrolyzed was poured into the tube. A glass stirring rod driven at about 300 r.p.m. extended vertically down into the solution almost to the cathode.

For the tests below 20 ma., a platinum anode was connected to the solution through a salt bridge. For the higher current tests, a 1 × 40 mm. platinum wire anode was dipped directly into the solution. Two salt-bridge probes were used, each connected to a separate saturated calomel half-cell of about 50 sq. cm. area. The electrode of one calomel cell was connected to the regulator, and the measuring instruments were connected between the second calomel cell and the "cathode" in order to minimize any effect of the instruments on the behavior of the regulator.

In every case, the end of the salt bridge from the probe should be placed close to the "cathode" in order to avoid measuring any of the resistance drop component of voltage across the solution. If possible, its ends should be turned upward to avoid collection of bubbles. When bubbles accumulate at the end of, or in, the salt bridge, they greatly increase its resistance and thereby cause poor regulation.

Variations in the cathode-solution potential were studied with three instruments:

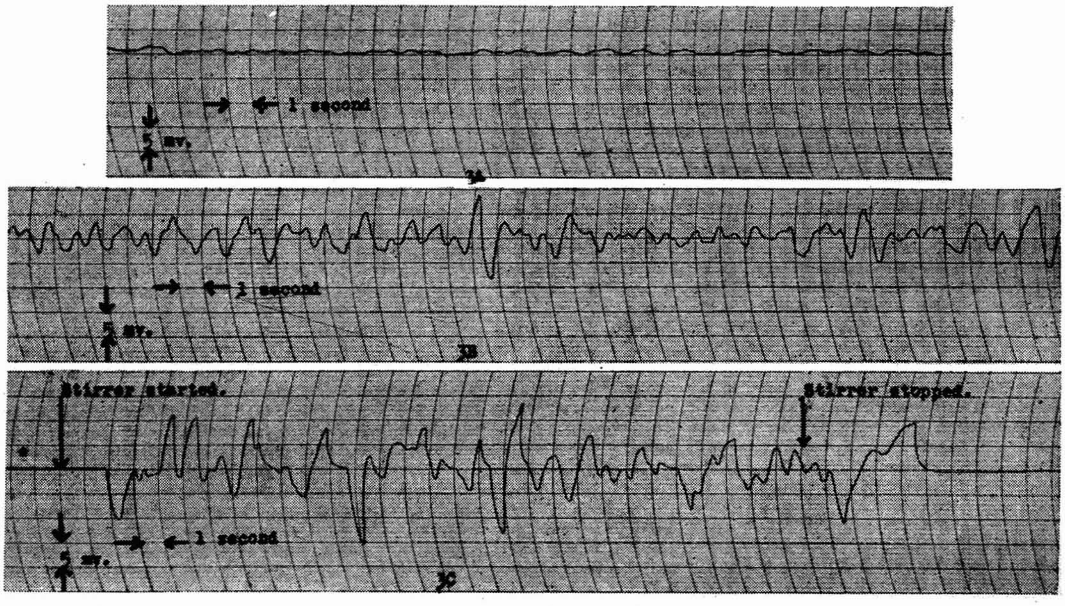

Figure 3. Records of Cathode-Solution Potential Variations, Cathode Negative

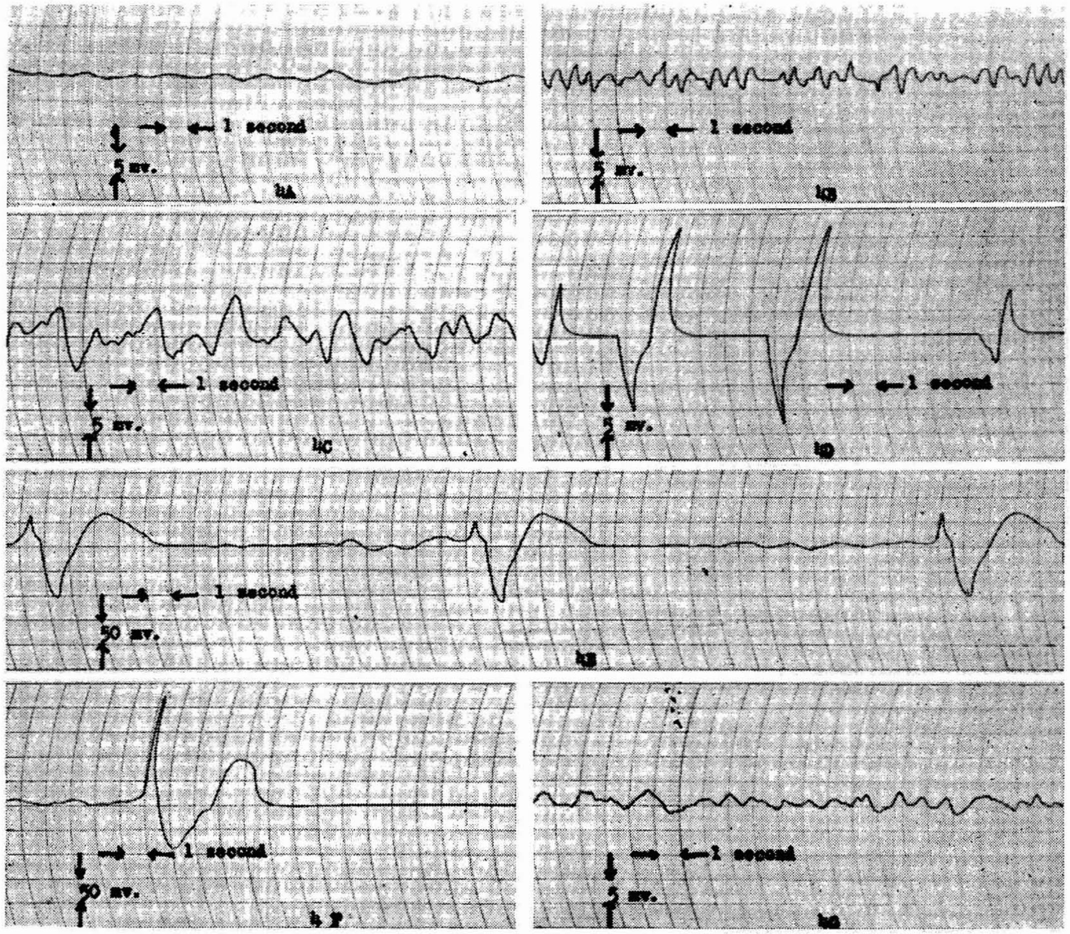

Figure 4. Records of Cathode-Solution Potential Variations, Cathode Negative

1. A potentiometer capable of measuring steady direct current potentials to 0.5 mv. This was used to detect drift. No drift was ever detected except on overnight tests and then it was always less than 1 mv.

2. A Brush, Type BL211, alternating current amplifier and recording oscillograph. This device had a constant input impedance of 10 megohms resistance. It could record accurately variations over a frequency range from 0.2 to 100 cp. with a sensitivity of up to 1 mm. of chart deflection per mv.

3. A Dumont, Type 208B, cathode-ray oscilloscope. It had an input impedance of 2 megohms resistance and 30 mmfd. shunt capacity, and would respond faithfully over a frequency range from 2 to 100,000 cp. It was always used at full gain, where the sensitivity was ca. 2 mm. per mv. This instrument was used to observe visually any erratic variations in cathode-solution potential, particularly above 100 cp. where the recording oscillograph was ineffective.

Results. In the vast majority of cases, the regulation was excellent. For cathode-solution potentials between +1.5 and −1.5 volts and for current ranging from 0.1 to 500 ma., control was often better than ±2 mv. A typical oscillogram is shown in Figure 3*A*, and the conditions are listed in Table I.

Regulation was slightly affected by changing from an external "anode" (resistance of salt bridge was approximately 2000 ohms) to an internal anode. With the internal anode, regulation to ca. ±6 mv. was obtained, though it could be improved by reducing the voltage across the Variac to 6 volts or less. The reason for the wider variation lies in the fact that the current-carrying potentiometer (Type 200B Variac) has a finite number of turns, and introduces discontinuities of about 5 mv. when connected to a

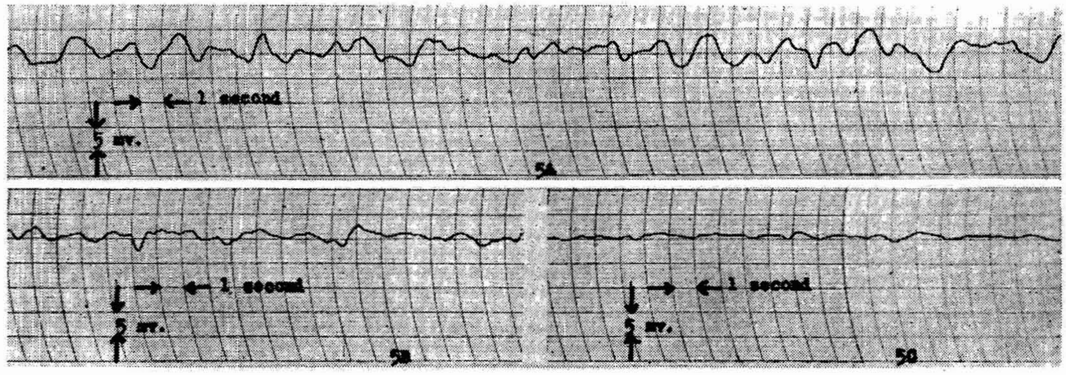

Figure 5. Cathode-Solution Potential Variations, Cathode Positive

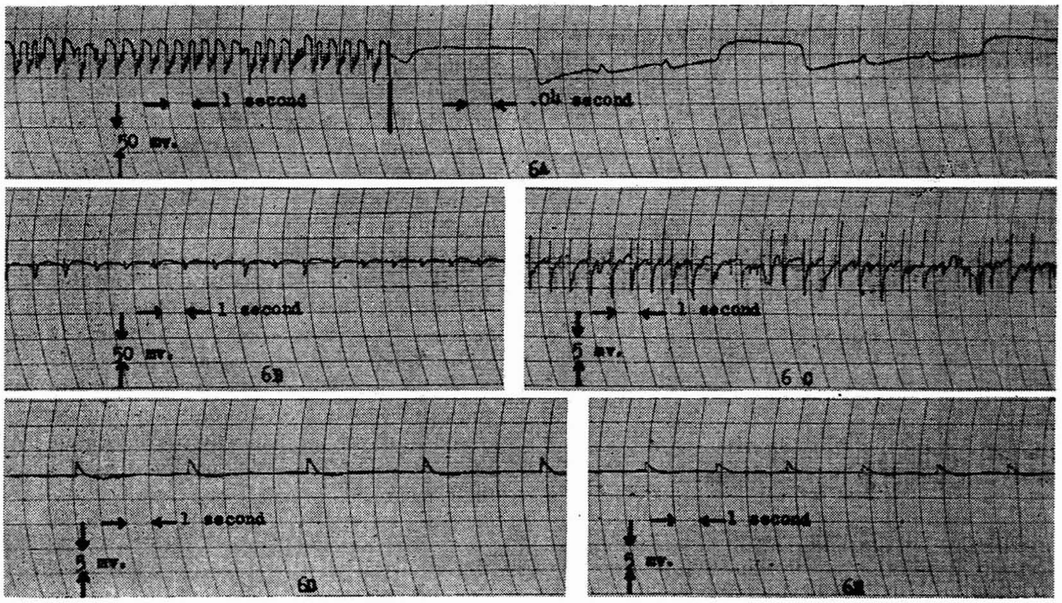

Figure 6. Cathode-Solution Potential Variations

Tantalum cathode in 1 *M* perchloric acid

12-volt plating battery. When a high resistance such as a salt bridge is in the anode circuit, the fraction of the discontinuity that appears between the cathode and probe is appreciably smaller than when an internal anode is used.

The regulator was helpful in decreasing the magnitude of slow changes in potential that resulted from stirring the solution. A comparison of Figure 3*B* and *C* also shows that the fluctuations were directly the effect of stirring. That was generally found to be the case, except for tantalum electrodes, discussed below. For all oscillograms, time increases toward the right, and upward deflection indicates a change toward more positive cathode-solution potential.

Figure 4 illustrates a series of experiments starting at a slightly negative potential, where the anode current and cathode-solution potential fluctuations were very small. When the cathode was made more negative, a region covering about 0.5 volt was found where regulation was very poor. At still more negative potentials regulation was regained. In an effort to examine the nature of the phenomena that resisted regulation, changes in the cathode-solution potential were studied at a number of impressed voltages. The effects of stirring and electrode material on the phenomena were examined simultaneously.

The oscillograms shown in Figure 4 were taken in stirred solutions with the regulator off. Changes in potential were too rapid to allow any effective control by the instrument. In addition to the general trend of the pattern illustrated by Figure 4, the direction of the initial change is reversed in going from *D* to *E*, and the fluctuations become regular in size and in periodicity. In going from *E* to *F*, the individual peaks become larger but the time interval between peaks becomes longer. Figure 5 presents similar evidence in which the polarity of the "cathode" was changed to positive. A region of instability was found, but the fluctuations were very much smaller.

The importance of the past history of the electrode was clearly evident. In several experiments, the potential on a platinum electrode in contact with a solution of cyanide was very steady for several hours. Generally, however, the sharp fluctuations began to appear about 30 minutes after electrolysis began, and on several occasions they appeared almost immediately.

Experiments with tantalum, tungsten, and rhodium confirmed the importance of the electrode history, but this discussion is limited to a few experiments with tantalum electrodes. Oscillograms with a new cathode at a particular potential could be gravely affected by a momentary reversal of cathode polarity, by scratching the electrode surface, or by approaching the potential from a different direction. The speed and periodicity of the fluctuations were very marked, and characteristically different from those obtained with platinum in contact with cyanide. Using the cathode-ray oscilloscope, uniform periodic fluctuations of 2- to 10-mv. amplitude and of the order of 1000 cp. were often observed in addition to those that could be detected by the recorder. At any specific potential the frequency increased and decreased irregularly, although sometimes it remained fairly constant for intervals of several seconds.

The behavior of tantalum was also characterized by the possibility of attaining very large positive cathode-solution potentials (up to 6 volts, which was the limit of the authors' instrument) without passing anode currents greater than 0.5 ma. However, such behavior is consistent with the rectifying properties of tantalum as observed by others (*3, 7*). After the surface of the electrode was scratched with a glass rod, the current increased more than tenfold, but the fluctuations in potential increased only about 50%. However, it was still possible to reach unusually high potentials. At negative cathode-solution potentials, the tantalum behaved normally, and a maximum potential of −1.5 volts was reached. It seems probable, therefore, that the high oxygen overvoltage was due to the presence of a nonconducting coat of oxide on the tantalum surface.

The oscillograms in Figure 6 are typical of those encountered with tantalum and were obtained within a few minutes of one another in the same order in which they appear in the figure. Between *D* and *E*, the cathode-probe potential was made more negative in steps of 1 volt. Conditions were very steady between +3.0 and −1.0. At −1.1 volts the potential reached its maximum instability on the negative side, but the variations were tiny compared to those found on the positive side.

The preceding studies are of particular importance in the electrodeposition of radioactive tracers, where it often happens that the total amount of depositable element involved in the electrolysis is insufficient to cover an "inert" electrode with as much as a monolayer of deposit (*11*). In all electrolyses involving the deposition of macroamounts of an element, regulation was very

Table II. Current-Voltage Relationships Encountered with New Tantalum Electrode in Contact with 1 *M* Perchloric Acid

Setting	V_{CP}	$-I_A$	$-V_{AC}$	Remarks
1	0.75	<0.1	0.8	Steady readings
2	1.30	<0.1	1.3	Steady readings
3	1.75	<0.1	1.8	Steady readings
4	2.00	0.2	2.7	Steady readings
5	2.15	1.0	3.9	Steady readings
6	2.30	1.2	4.1	Steady readings
7	2.50	0.6	3.8	I_A and V_A initially increased, dropped suddenly, then drifted lower very slowly
8	2.65	0.3	3.5	Similar to No. 7
9	2.90	0.3	3.8	Similar to No. 7
10	3.10	0.4	4.2	Small increase in I_A followed by slow drift backward
11	3.30	1.2	5.3	Similar to No. 10
12	3.50	2.8	7.4	Similar to No. 10

satisfactory. However, in an effort to record the regulation under the worst possible conditions, the following experiment was performed:

> Using the regular platinum cathode and an internal platinum anode in the form of a long wire 1 mm. in diameter, the deposition of copper from a solution of acidified cupric sulfate was observed to proceed with excellent regulation when 0.5 ampere was passing, even though gases were being evolved rapidly at both electrodes. When the wire anode was raised slowly out of the solution, the current remained essentially unchanged, but the fluctuations grew larger. When only about 2 mm. of the wire anode were dipping into the solution, the severe fluctuations shown in Figure 7 were recorded.

DISCUSSION

From the data presented above, it appears that there are two, possibly three, phenomena leading to fluctuations in the cathode-solution potential. In the experiments involving a platinum electrode in contact with a solution of cyanide, the greatest fluctuations in potential occurred in a region just prior to the vigorous evolution of hydrogen. One can imagine the formation of uniform gas film having a high electrical resistance over the surface of the electrode. Breaking the film to form a bubble of gas would leave bare surface, lowering the resistance and allowing more hydrogen to be formed. In a stirred solution the bubbles would be liberated less regularly but more frequently. Oscillograms obtained by the authors nearly always confirmed that expectation.

Some of the fluctuations encountered in working with tantalum also appeared to be due to bubbles. On several occasions after the surface had been covered with many bubbles of assorted sizes, the fluctuations occurring at moderately positive potentials could be eliminated entirely by tapping the cathode support sharply. At first a very large peak on the oscillogram coincided with each tap, but the surges grew smaller and finally disappeared after several minutes of tapping. Even though the fluctuations had disappeared, many bubbles were still visible on the electrode surface.

The fluctuations in potential which were obtained at high current densities (Figure 7) can be explained by variations in the high resistance of the gas film and layer of bubbles around the anode. By placing the stirrer closer to the anode (and farther from the cathode), fluctuations were very much reduced in magnitude.

Once with the platinum-cyanide system, and twice with the tantalum-perchlorate system, a distinct maximum was discovered in the anode current upon increasing the cathode-solution potential manually, and waiting about 2 minutes at each potential before taking readings. The maximum was not detectable when the potentials were changed in the opposite direction. The data shown in Table II are typical and were repeated three times in succession without difficulty. After the electrolysis had proceeded for about 30 minutes, the maximum disappeared. It seems likely, therefore, that the increased instability of potential which sometimes occurred upon using a regulator may have been due to corrective action of the slide wire in the wrong direction as a result of the anomalous relationship between changes of potential and current. In fact, the adverse effect of using the regulator in such situations can be decreased by gearing down the Brown motor to a much slower rate of corrective change.

The oscillograms presented in this paper indicate that there may often be very large fluctuations in the cathode-solution potential which would be underestimated or completely overlooked if an ordinary voltmeter were employed. In the case of a potentiometer, the galvanometer would show erratic excursions in both directions from the balance position, but would give little indication of the true magnitude or frequency of the voltage fluctuations because of inertia of its pointer. In every case where the regulator was used, the average value of the cathode-probe potential remained constant and equal to the regulator setting, and the fluctuations that occurred were about this average value. These reasons probably explain why previous users of slow regulators have not reported these fluctuations in more detail. It is possible that a fast regulator such as one described by Heyd, Ohmart, and Hites (*4*, *5*) would be able to eliminate the rapid as well as the slow fluctuations in cathode-solution potentials except in situations similar to that in Table II, where the relation between changes in potential and current reverses its sign.

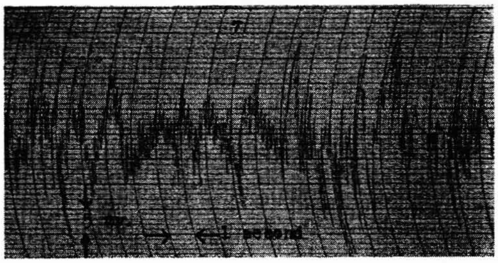

Figure 7. Cathode-Solution Potential Variations

Internal anode and high current

ACKNOWLEDGMENT

The authors wish to thank C. B. Pickle for providing the basic design of the instrument, and D. B. Ehrlinger and J. C. Griess, Jr., for carrying out many of the preliminary tests.

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High-Frequency Oscillator

Utilizing Heterodyne Principle to Measure Frequency Changes Induced by Diverse Chemical Systems

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A high frequency oscillator utilizing the heterodyne principle has been designed for use in measuring frequency changes induced by diverse chemical systems. The increased sensitivity obtained can be utilized to detect and measure concentration changes of a nature that would require long and tedious procedures by ordinary chemical methods.

HIGH-frequency oscillations have been used for almost 40 years to determine dielectric constants, which have served to indicate the course of reactions, to follow the progress of fractional distillations, to determine the composition of binary mixtures of certain isomers, and in some cases to determine the moisture content of solids (*1*, *5*). However, the direct application of high-frequency oscillations to chemical analysis has received little attention until very recently.

In 1946 Jensen (*4*) reported a high-frequency oscillation titrator applicable to the performing of conductometric titrations without the use of standard-type electrodes. The conductometric cell is placed inside the field of the tank circuit coil of a high-frequency oscillator and the change in plate current of the oscillator tube is plotted against volume of the titrating reagent added to the cell. The reversal of the curve of plate current versus volume indicates the equivalence point of the titration. In 1947 Blake (*2*) reported the use of radio-frequency oscillations in making conductometric titrations. In the Blake apparatus the output of a radio-frequency oscillator is coupled inductively to a metering system where the radio-frequency current is rectified and the direct current registered on a meter. The titration cell is placed inside the core of the coupling transformer and the rectified current is plotted against volume of titrating reagent. Reversal of the curve of current versus volume indicates the equivalence point.

The instrument designed by the present authors is based upon the principle that the frequency of an oscillator changes with a change of composition of the core material of the tank circuit coil. In this instrument, of the heterodyne type, the tank circuit coil form is hollow and the core material is changed by placing a small glass cell, filled with any desired chemical system, within the hollow coil form. The frequency change is plotted as a function of concentration. Because frequency changes as small as 10 in 4,000,000 cycles may be measured accurately, the increased sensitivity thus obtained can be utilized to detect and measure concentration changes that would require long and tedious procedures by ordinary chemical methods.

EXPERIMENTAL

The heterodyne analyzer consists essentially of two high-frequency oscillators (one a "reference" and the other a "working" oscillator), and a system for measuring the frequency difference between the two oscillators. The purpose of the reference oscillator is to make possible a means of measuring the frequency change of the working oscillator without measuring the actual frequency. By beating the output of the working oscillator with that of a reference oscillator, a difference (beat) frequency in the audio range may be obtained. A change in frequency of the working oscillator will thus cause a frequency change of equal magnitude in the beat frequency. This beat frequency change can be measured with a satisfactory degree of accuracy. The block diagram (Figure 1) illustrates the manner of operation of the analyzer.

Interchange of energy between the two oscillators through the mixer tube is prevented by the two buffer stages. The output of each oscillator is fed into its buffer stage and thence into the mixer tube. No amplification is gained in the buffer stage; rather, the buffer serves solely to isolate completely the sources of oscillation from each other, so that a change in frequency of the working oscillator cannot affect the reference oscillator. The output of the mixer stage, consisting of the two original frequencies, the sum, and the difference of the original frequencies, is conducted to the detector system where the beat frequency is isolated. The beat frequency is then amplified in the audio amplifier stage and can be measured by any standard frequency-measuring device.

In this laboratory an accurately calibrated audio-oscillator (Hewlett-Packard 200-I) and an oscillograph (DuMont 208-B) were used. The output from the audio-amplifier stage was fed to the vertical plates of the oscillograph while the output of the audio-oscillator was fed to the horizontal plates. The frequency of the audio-oscillator was varied until the Lissajous pattern on the screen of the oscillograph indicated that the frequency of the audio-oscillator was equal to the heat frequency. The choice of an audio-oscillator–oscillograph combination for a frequency meter was purely expedient. A number of commercial direct reading audio-frequency meters could have served just as satisfactorily.

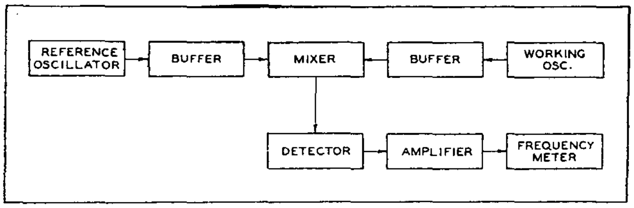

Figure 1. Block Diagram of Heterodyne Analyzer

The change in frequency of the working oscillator, caused by insertion of a chemical system into its field, is measured in the following manner:

Under normal stand-by conditions (no chemical system within the field of the working oscillator) the frequency meter indicates the difference (beat frequency, stand-by) between the frequencies of the working and reference oscillators. If a chemical system is then placed inside the tank coil of the working oscillator, the frequency of this oscillator will change while the frequency of the reference oscillator is unaffected. The frequency meter will then read the difference (beat frequency, loaded) between the frequencies of the reference oscillator and the working oscillator with a chemical system in its tank coil. The difference between the two meter readings (beat frequency, stand-by minus beat frequency, loaded) is equal to the change in frequency of the working oscillator.

One of the most important physical characteristics of the instrument is the frequency stability of the two oscillators. The

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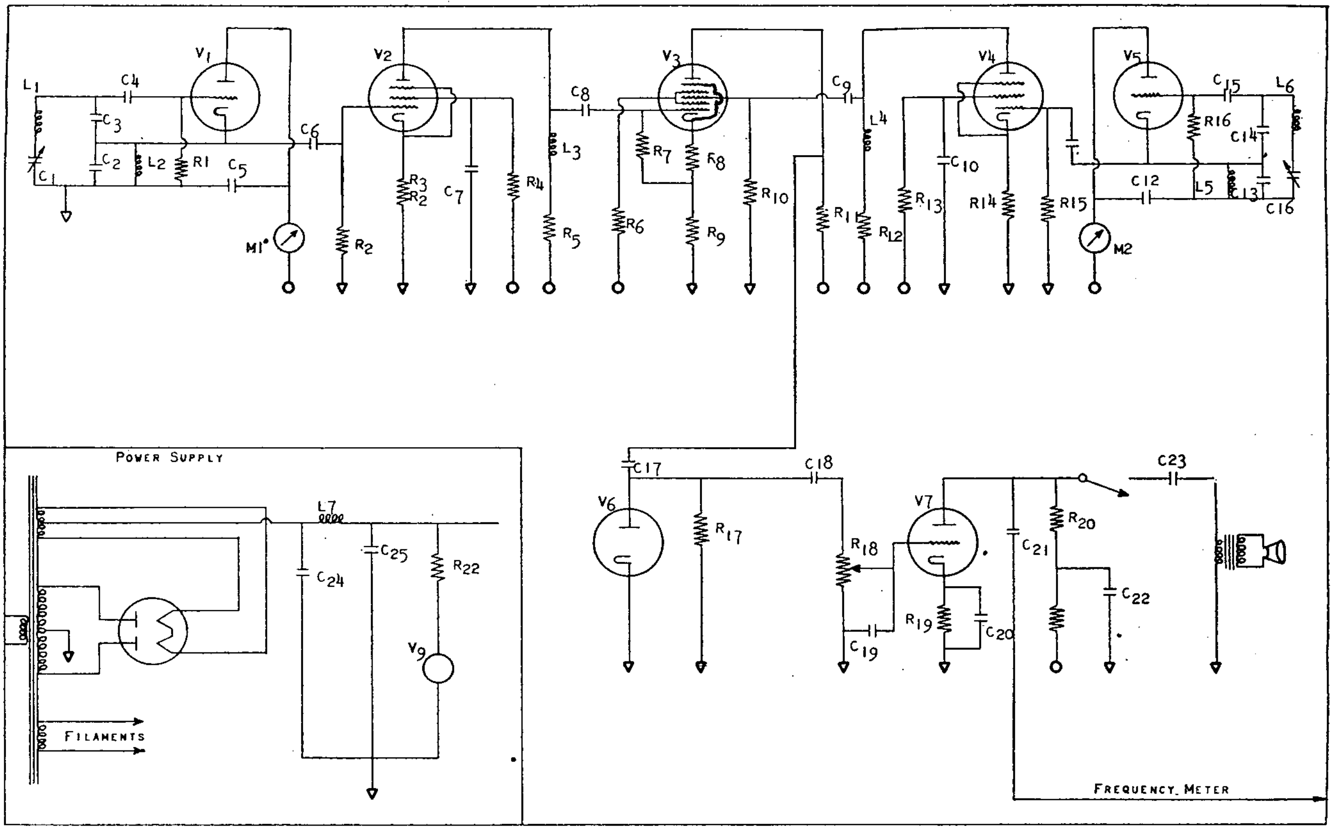

Figure 2. Circuit Diagram of Heterodyne Analyzer

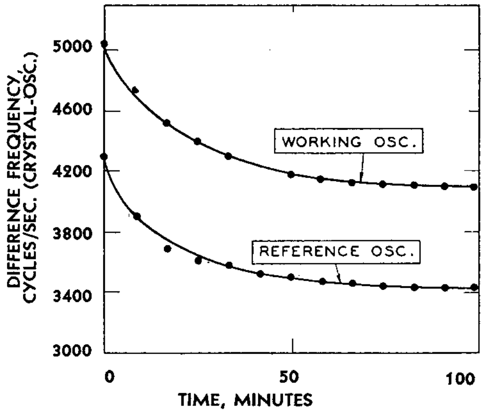

Figure 3. Frequency Drift of Each Oscillator

two oscillators under stand-by conditions must be able to maintain a constant frequency difference. The circuit diagram for the oscillators (Figure 2) is the Clapp modification of a Colpitts-type oscillator and is especially well suited for work of this kind. A description of the individual parts is given in Table I. The oscillator circuit is described by Gramer (*3*), who investigated the stability of the oscillator in the QST laboratories, and reports that the circuit "results in an oscillator whose frequency is almost independent of plate voltage and tube thermal effects." The unusual stability is due to loose coupling between the oscillator tube and a high Q tuned circuit. The large capacity of C_2 and C_3, compared to C_1, makes coupling between the tube and the tuned circuit very loose, so that the circuit Q can be kept very high; in addition, these large values "swamp" the grid to cathode and plate to cathode capacities to such an extent that the effect of any change in these capacities becomes negligible.

The stability of the oscillators in the heterodyne analyzer is shown in Figures 3 and 4. Figure 3 shows the frequency drift from a cold start of each oscillator relative to that of a 4.3-megacycle crystal-controlled oscillator. The output of each oscillator independently was beat with the output of the previously warmed up crystal oscillator. The difference frequency was measured

Table I. Values of Component Parts of Analyzer

Condensers		Resistors	
Condenser No.	Value, mfd.	Resistor No.	Value, K.[a]
C_1	150 mmfd.	R_1	100
C_2 and C_3 (Silver	0.001	R_2	750
C_4 Mikes)	100 mmfd.	R_3	400 ohms
C_5	0.1	R_4	250
C_6	0.001	R_5	250
C_7	0.01	R_6	5.6
C_8	0.002	R_7	150
C_9	0.002	R_8	330 ohms
C_{10}	0.01	R_9	680 ohms
C_{11}	0.001	R_{10}	500
C_{12}	0.1	R_{11}	600
C_{13} and C_{14} (Silver	0.001	R_{12}	250
C_{15} Mikes)	100 mmfd.	R_{13}	250
C_{16}	150 mmfd.	R_{14}	400 ohms
C_{17}	500 mmfd.	R_{15}	750
C_{18}	0.06	R_{16}	100
C_{19}	50 mmfd.	R_{17}	560
C_{20}	0.02	R_{18}	150
C_{21}	0.02	R_{19}	1200 ohms
C_{22}	0.1	R_{20}	40
C_{23}	0.02	R_{21}	50
C_{24} and C_{25}	8	R_{22}	7

Coils		Tubes	
Coil No.	Value, mh.	Tube No.	Type
L_1	0.006	V_1	6J5
L_2	2.5	V_2	6K7
L_3	2.5	V_3	6L7
L_4	2.5	V_4	6K7
L_5	2.5	V_5	6J5
L_6	0.006	V_6	6H6
L_7	8 henries	V_7	6J5
		V_8	5U4G
		V_9	V-R 150

[a] 1000-ohm units.

every minute for 96 minutes. Because the crystal oscillator has negligible drift, the variation of the difference frequency is due to the drift of the oscillator. The two curves are almost parallel throughout their length and after 45 minutes show practically zero drift and a constant frequency difference. Figure 4 is a plot of time versus beat frequency, stand-by from a cold start. During the first 45 minutes the beat frequency gradually increases, then slopes off and becomes constant within 10 cycles. The frequency of each oscillator varies about 1 cycle per volt with fluctuations of line voltage, but the frequency difference is unaffected.

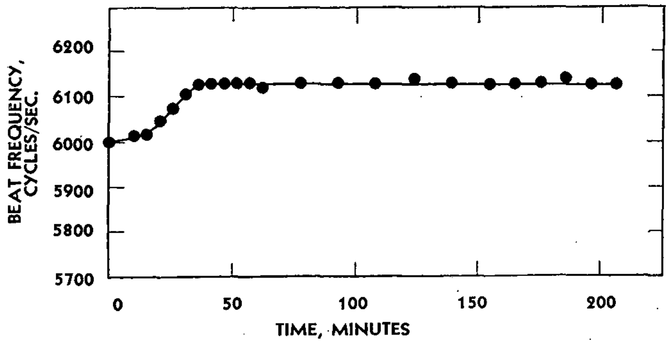

Figure 4. Time *vs.* Beat Frequency

The buffer, mixer, detector, and amplifier stages are all of standard design and may be modified in any way necessary to fit a particular need. The buffer is an R-C coupled 6K7 R-F amplifier. The mixer stage consists of a 6L7 tube with a split cathode resistor to maintain proper bias on each of the control grids. A diode-type detector circuit is employed utilizing a 6H6 tube. The audio-amplifier uses a 6J5 tube with the component values adjusted to give approximately a linear amplification factor of 14 over a frequency range of 500 to 5000 cycles.

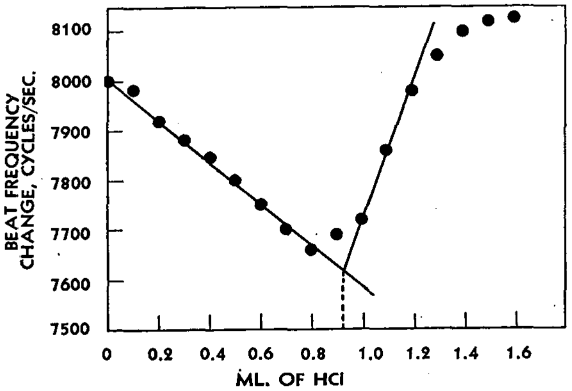

Figure 5. Acid-Base Titration

The layout and mechanical construction of a high-frequency oscillator of exceptional stability are at least partially an art. Stray wire capacity, chassis currents, radiation effect, etc., constitute individual problems with every set. The technician who has had no previous experience with high-frequency oscillators will meet with some difficulty in his first attempt. However, any radio amateur or veteran with radio-radar maintenance experience should have no difficulty in obtaining the required stability.

APPLICATIONS OF THE ANALYZER

The analyzer is adaptable to most types of conductometric titrations. Acid-base or precipitation titrations can be made readily. A common 50-ml. heat-resistant test tube (25 by 105 mm.) fits snugly into the coil form. The titrant is added from a microburet, the beat frequency change is measured after the addition of each increment, and a plot is made of volume versus beat frequency change. Figure 5 is a sample graph made by this method. Ten milliliters of 0.01000 N potassium hydroxide in the test tube were diluted to approximately 30 ml. and titrated with 0.1060 N hydrochloric acid.

Table II. Beat Frequency Change Induced by Various Organic Compounds

Compound	Beat Frequency Change, Cycles/Sec.
n-Pentane	660
n-Hexane	681
n-Heptane	697
Methanol	5560
Ethanol	4763
n-Propyl alcohol	4288
Isopropyl alcohol	4117
n-Butyl alcohol	3895
Isobutyl alcohol	3906
sec-Butyl alcohol	3658
tert-Butyl alcohol	2998
n-Amyl alcohol	2031
tert-Amyl alcohol	2938
n-Octyl alcohol	2550
Acetone	4397
Methyl ethyl ketone	4103
Diethyl ketone	3922
Methyl *n*-amyl ketone	3062
Acetophenone	4014
Benzene	810
Toluene	830
Bromobenzene	1675
Aniline	2040
Nitrobenzene	5720
Acetic acid	2190
Propionic acid	1242
Butyric acid	1094
Caproic acid	1005
Methyl acetate	2135
Methyl *n*-propionate	1890
Methyl *n*-butyrate	1687

By using a small volumetric flask (special design, 19-ml. capacity) which fits entirely inside the coil form and a previously prepared calibration chart, direct concentration measurements can be made on many binary organic systems. The calibration curve of composition versus beat frequency change is nearly linear and for many systems is sufficiently steep so that accuracy of the order of 0.2 to 2.0% can be attained. A calibration chart of the aniline-nitrobenzene system shows that the curve is a straight line with a slope of 36 allowing an accuracy 0.3%.

The slope of the calibration curve is determined by the difference in the beat frequency change induced by the pure components. The greater the difference in the beat frequency change induced, the steeper the slope of the calibration curve and the greater the attainable accuracy. The beat frequency changes induced by a number of organic compounds have been measured and are listed in Table II. A difference of beat frequency change equal to 5000 cycles between any two compounds represents an attainable accuracy of 0.2%.

ACKNOWLEDGMENT

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Polarographic Microdetermination of Chromium in Dusts and Mists

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A polarographic method for the determination of chromium in dust and mists is described. The chromium may exist in the form of its ores, oxides, and various inorganic salts. Samples analyzed are of a micro or semimicro size. The amounts of chromium found vary from a few micrograms to several milligrams. A number of factors influencing the accuracy of the determination have been studied. Results obtained with the proposed method are compared with results obtained with standard methods. Analyses of several National Bureau of Standards samples are also given.

THE determination of chromium in the air of a plant manufacturing chromate salts and liquor concentrates from chrome ore presents a variety of problems.

The manufacturing process begins with the crushing and grinding of the ore to less than 200-mesh size. The pulverized ore is mixed with sodium or potassium carbonate and some dolomitic lime. The mixture is heated to a high temperature in rotary kilns where most of the chromium is converted to the chromate form. The kiln products are transported to large leaching tanks, and the soluble components are removed with water. The highly alkaline leach is gradually neutralized with sulfuric acid; aluminum, silica, and various sulfates are removed by pH control, concentration, and filtration. The chromate is converted into the dichromate at a pH of approximately 4, and the product is shipped either as a concentrated liquor, or after further concentrating, crystallizing, filtering, drying, and bagging, as the dichromate salt itself.

Some of these operations are dustier than others; some may not be as dusty but may contain greater percentages of chromium in one form or another. In plants where most of the operations are carried on under one roof, the air may contain the products of all the operations, with possibly a larger proportion of the products of the operation nearest at hand. In this investigation, it was desired to determine the total amount of chromium in the air and to get estimates of the water-soluble chromium compounds, the chromium in which was mostly hexavalent with traces of the tri- and divalent forms.

Of the various analytical methods available, it was thought that a polarographic method (*6*) would probably be the more easily adaptable to a rapid routine procedure.

Thanheiser and Willems (*10*) reported a polarographic method for the determination of chromium in steel. A 0.1- to 0.2-gram sample was dissolved in hot concentrated perchloric acid, neutralized, oxidized in alkaline solution with hydrogen peroxide, boiled, and then run polarographically in approximately 1.6 *N* sodium hydroxide. Determinations of 0.5 mg. and less of chromium in 50 ml. of final solution were said to be run only with great difficulty. Molybdenum, tungsten, vanadium, and aluminum were reported as noninterfering. Stackelberg, Klinger, Koch, and Krath (*9*) also reported a method for analyzing for chromium in steel by use of the polarograph. In their method a 0.2-gram sample was dissolved in 1 to 1 hydrochloric acid, oxidized with nitric acid, dried, fused with 4 grams of sodium peroxide, dissolved in hot water, and diluted to 100 ml., and a portion of the supernatant liquid was run polarographically. The final solution was between 1.5 and 2.0 *N* in sodium hydroxide at the time the polarogram was run. No standardization figures were given, but comparative analyses were shown on a number of steel samples.

Lingane and Kolthoff (*7*) studied the reduction of the chromate ion at the dropping mercury electrode in unbuffered, buffered, and strongly alkaline media. They found that in a 1 *N* sodium hydroxide solution the half-wave potential is practically constant and independent of the chromate concentration, and that the diffusion current is directly proportional to the concentration of the chromate ion. It remained to develop techniques to analyze chrome ore and chromium compound mixtures and to determine much smaller quantities of chromium. The procedure as finally worked out consists of two parts: one for solutions and one for residues, the latter essentially a fusion method.

REAGENTS AND SOLUTIONS

Unless otherwise specified, all reagents are of analytical quality.

Standard Chromate Solution. Potassium dichromate obtained from the National Bureau of Standards (2.829 grams) is dissolved in double-distilled water and made up to 1 liter. One milliliter of this solution is equivalent to 1 mg. of chromium. Solutions containing 100 and 10 micrograms of chromium per milliliter are made by diluting this stock solution with double-distilled water.

Sodium Hydroxide Solution. Depending upon the assay, 42 to 45 grams of reagent quality sodium hydroxide per liter of final solution are dissolved in freshly boiled double-distilled water, cooled, and made up to volume. This should be stored in a bottle (preferably of borosilicate glass) from which carbon dioxide and water are excluded, and from which the solution is withdrawn without disturbing the carbonates, silicates, and other insolubles which settle to the bottom of the bottle. The solution may be standardized and adjusted to exactly 1 *N;* however, slight variations in normality do not greatly affect the results. To obtain a uniformly consistent blank, it is desirable to make several liters of this solution at a time.

Fusion Mixture. Four parts by weight of magnesium oxide to one part of sodium carbonate are mixed thoroughly, dried, and stored in a dustproof bottle. It is mixed thoroughly each time before using.

Distilled Water. Ordinary distilled water does not contain noticeable amounts of chromium. However, there is always a possibility that the water may contain free chlorine, organic matter, or other volatile substances which may react with the chromate ion. Once the chromium is oxidized it is necessary to use either freshly boiled distilled water or double-distilled water that has been well kept.

Mercury. The mercury is first cleaned by letting it fall several times through a column of 10% nitric acid. After rinsing and drying it is triply distilled in an all-glass apparatus at a pressure of 20 mm. with a very fine stream of air sweeping through and over the mercury throughout the distillation.

INSTRUMENTATION

A Model XXI Sargent pen recording polarograph was used, which had an ultimate sensitivity of 0.003 microampere per millimeter scale deflection. Diffusion currents, i_d, were obtained by taking the averages of the oscillations of the residual and the limiting diffusion currents. The supplementary damping provided on the instrument was not used even at high concentrations of chromium. One capillary was used throughout the entire problem. It had a drop time of 5.80 seconds, and the amount of mercury, *m*, passing through the capillary in a 1 *N* sodium hydroxide solution with an open circuit was 1.06 mg. per second. With a closed circuit and an imposed e.m.f. of −0.85 volt *vs.* the saturated calomel electrode (S.C.E.), the drop time was 5.52 seconds and m was equal to 1.09 mg. per second. The mercury column height was kept at 20 inches (50 cm.) and the temperature at 25° ± 0.2° C. (*1*). All samples were bubbled for at least 15 minutes with high purity nitrogen gas passed through 1 N sodium hydroxide before passing through the samples. Small, simplified polarographic cells (Figure 1) were used. They required small mercury pool anodes; and anodic contact was made by means of a beaker partially filled with mercury and suspended in a constant temperature bath. These cells were designed to be easily changed after an analysis. Although the use of mercury pool anodes in place of the usual saturated calomel electrode requires a relatively large amount of triply distilled mercury, the possibility of contamination of one sample by another and the bother of cleaning and keeping salt bridges are eliminated.

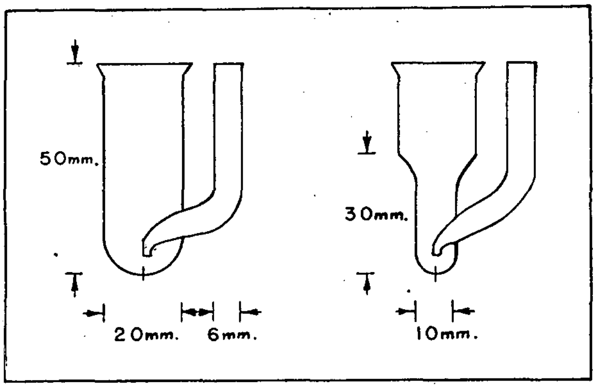

Figure 1. Simplified Polarographic Cells

Glassware. All glassware was cleaned with Calgonite, rinsed with tap water, rerinsed in 1:1:8 solution of nitric and sulfuric acids in water, and finally rinsed with distilled water. The common sulfuric acid–chromic acid cleaning solution was rigorously avoided.

COLLECTION OF DUSTS AND MISTS

A sufficient quantity of air is drawn through one of the sampling devices recommended for dust and/or mists (*3*, *5*, *8*) to give a sample containing 5 or more micrograms of chromium. A 15-cubic foot sample of air is usually ample. During the course of this investigation several sampling devices were used. These included the Greenburg-Smith impinger with 100 ml. of either distilled water or 1 N sodium hydroxide, midget impingers with 10 ml. of distilled water, a 5.5-cm. Eaton-Dikeman No. 623-026 filter paper in a Casella filter paper holder, a 15-cm. E. & D. No. 623-026 filter paper in a special filter head, and a Mine Safety Appliance Company (M.S.A.) electrostatic dust and fume sampler. The M.S.A. midget impinger hand pump set to draw 0.1 cubic foot per minute was used for the midget impingers. A Wilson portable electric vacuum pump, drawing air at the rate of 1 cubic foot per minute, was used for the impingers and the small filter paper holders, and a Crowell pump, size 2A, driven by a gasoline motor and drawing air at a maximum of 13 cubic feet per minute, was used for the large filter paper holders. Air volumes of all except the midget impingers were measured by means of specially adapted orifice meters which had been previously calibrated against a wet gas meter.

Sampling time varied from 2 to 30 minutes within the plant and from 2 to 4 hours at distances up to 1 mile from the plant. A number of bulk samples were obtained by means of a household-type vacuum cleaner run continuously for periods up to 72 hours. Settled rafter dust samples were also collected. However, the usual sample was obtained in 15 minutes at a rate of 1 cubic foot per minute.

ANALYTICAL PROCEDURE

Solutions. When the contaminated air is sampled with an impinger device (*5*, *8*) distilled water is preferred as a collecting medium. The solution is filtered through a fine-textured filter paper such as Whatman No. 42, and the filtrate is collected in a 250-ml. beaker. A small amount of acetone should be used to free the impinger of particles of grease or oil adhering to the sides. The filter paper and the container are thoroughly washed with a fine stream of distilled water. The filtrate, which contains most of the soluble and slightly soluble chromium compounds, is neutralized, if acid, with 1 N sodium hydroxide solution. At this point the volume of the solution should be between 150 and 175 ml. One milliliter of 1 N sodium hydroxide to excess is added, the solution is stirred, and 1 ml. of 30% hydrogen peroxide is added. The beaker is covered with an elevated watch glass, and the solution is brought to a quick boil, boiled strongly for a few minutes, and then evaporated to near dryness. The beaker is removed from the hot plate, the cover and sides are washed down with freshly boiled distilled water, and the solution is taken just to dryness without visible boiling—preferably with an infrared heater.

After cooling, 10.0 ml. of 1 N sodium hydroxide are added with a pipet and allowed to soak with intermittent stirring. If the solution is too cloudy it is centrifuged; otherwise some of the supernatant liquid is poured into a polarographic cell, a few drops of mercury are added, and the solution is bubbled for at least 15 minutes with purified nitrogen or hydrogen and run polarographically between 0.5 and 1.2 volts *vs.* the saturated calomel electrode. The half-wave potential rarely varies from −0.85 volt.

Residues. The chromium compounds remaining in the filter papers are analyzed by ashing the paper in a platinum crucible, preferably using a muffle furnace, starting from room temperature and heating to 550° C. After the paper is completely ashed, 100 to 200 mg. of fusion mixture are added and carefully and thoroughly mixed with the ash by means of an elongated glass rod drawn to a diameter of 1 to 2 mm. and fire-polished into a rounded point. The crucibles are covered and heated for 10 minutes between 825° and 850° C. After cooling, 10.0 ml. of 1 N sodium hydroxide are added with a pipet and allowed to soak for 0.5 hour with intermittent stirring. The supernatant liquid is centrifuged for 5 minutes, and some of the clear solution is added to a polarographic cell and run as above.

When the collecting medium is an alkaline solution, it may be treated similarly, except that precautions should be taken to keep the final concentration of the alkaline solution near 1 N. No additional sodium hydroxide need be added, and the solution is brought to near dryness rather than just to dryness. Other strengths of alkalinity may be used, provided that the standard curves (or the Ilkovič equation constants, *4*) are derived under similar conditions. The authors have on numerous occasions taken solutions containing 25 me. of sodium hydroxide almost to dryness and diluted with 25 ml. of water without causing too great a deviation from results obtained from a 1 N solution. The carbonates and silicates formed during the evaporation process do not noticeably interfere.

If filter paper is used as a collecting medium (*3*) and it is desired to find the amount of soluble chromium compounds, the filter papers are macerated in a beaker using a small volume of water. The water is filtered through a fine-textured filter paper by decantation. More water is added, stirred, and decanted. This process is repeated three to four times to obtain the greater part of the soluble compounds. The macerated paper is then transferred to the filter funnel and washed thoroughly at least six times with a fine stream of distilled water. The filtrate and residue are analyzed as given above. Should only the total chromium be wanted, the paper may be ashed directly.

Table I. Diffusion Current Values (Microamperes) Obtained by Using Routine Laboratory Procedures

Method	Chromium Taken, Micrograms									
	0	5	10	25	50	100	250	500	750	1000
Series A										
Untreated 1 N NaOH	0.01	0.09	0.16	0.41	0.79	1.48	3.86	8.00	11.3	15.4
solutions	0.01	0.09	0.15	0.40	0.80	1.54	3.92	7.76	11.3	15.0
Series B										
Treated as filtrates	0.02	0.07	0.15	0.44	0.82	1.52	3.92	7.76	11.4	15.2
	0.03	0.08	0.15	0.39	0.79	1.46	3.88	8.00	11.4	15.4
Series C										
Filtered and treated	0.01	0.06	0.22	0.28	0.64	1.28	3.40	7.52	10.7	15.0
as filtrates	0.03	0.04	0.09	0.31	0.65	1.40	3.32	...	...	15.2
Series D										
Treated as residues	0.01	0.08	0.16	0.36	0.85	1.54	4.11	8.20	...	16.2
	0.01	0.11	0.15	0.34	0.79	1.54	4.20	8.10	...	16.2

If the samples are collected by means of an electrostatic precipitator (*5, 8*), the dust in the tube is washed down with acetone or 95% alcohol, dried, picked up with water, and filtered if desired. Or the dried dust may be fused directly and run for total chromium.

STANDARDIZATION

Table I gives the diffusion currents obtained with four series of standards, each run by a different method.

Series A shows the diffusion currents, i_d, obtained from increasing concentrations of chromium, as the chromate ion, in 10.0 ml. of 1 *N* sodium hydroxide solution. These solutions were untreated except for bubbling with purified nitrogen before running polarographically. Using the m and t values obtained at the half-wave potential in 1 *N* sodium hydroxide solution, the calculated value for i_d/C is found to be 8.35 microamperes per millimole per liter. The observed average value obtained from Series A, Table I, after corrections for the blank, is 8.01 ± 0.16 microamperes per millimole per liter.

Series B shows the i_d values obtained by running the given amounts of chromium through the procedure as given for solutions, except that the filtration step was eliminated. The chromate was added to a 250-ml. beaker and diluted with 150 to 175 ml. of distilled water, 1 ml. of 1 *N* sodium hydroxide and 1 ml. of 30% hydrogen peroxide were added, and the solution was boiled, dried, etc. In Series C the given amounts of chromium were added to an air-sampling filter paper (5.5-cm. E. & D. No. 623-026) contained in a 150-ml. beaker. The paper was soaked for 0.5 hour and then filtered and run as outlined in the procedure. In Series D the given amounts of chromium were added to an air-sampling filter paper. The paper was ashed and fused as outlined in the procedure for residues.

The results are approximately, but not exactly, the same. Some difficulty is to be expected, and was encountered, in attempting to filter such small quantities of chromium from a comparatively large amount of paper. The results, however, are all of the same order of magnitude as the amount of chromium taken, and in the higher concentrations the losses are comparatively small.

The limit of sensitivity under carefully controlled conditions is approximately 0.05 microgram per milliliter of final solution. Under routine conditions a more practical lower limit is of the order of 0.2 microgram per milliliter of final solution. The usual final volume was 10.0 ml., but whenever small amounts of chromium were expected the final solution was reduced to 5.0 ml. of 1 *N* sodium hydroxide or less. Figure 2 shows the increased wave height which may be obtained from 5 micrograms of chromium by reducing the volume of the final solution. It must be recognized that the effects of other constituents in the sample are also increased. Nevertheless, amounts of chromium less than 1 microgram may be estimated in this manner.

Table II. Comparison of Analyses of Dusts and Chrome Refractory

Sample	Polarographic: Wt. of sample, mg.	Polarographic: % Cr	Macro Na_2O_2 Fusion, $KMnO_4$-$FeSO_4$ Titration, % Cr
Air-borne dust 1	0.83	17.6	17.46
Air-borne dust 2	0.84	14.6	14.98
Rafter dust 1	0.81	15.6	15.50
Rafter dust 2	0.97	16.7	16.28
Bureau of Standards 103	0.92	25.5	25.30[a]
	1.39	25.9	25.30[a]
	2.37	25.7	25.30[a]

[a] Certificate value converted to per cent chromium.

Table II gives comparative analyses of various dusts and the National Bureau of Standards sample of chrome refractory 103. The polarographic samples were weighed on an Ainsworth micro balance, mixed with fusion mixture, and run as outlined in the procedure for residues. Table III gives comparative analyses for some steel samples. The steels were dissolved in platinum crucibles with 1 to 1 hydrochloric acid plus a drop of concentrated sulfuric acid, brought to fumes of sulfur trioxide, oxidized with nitric acid, dried, and fused with magnesium oxide-sodium carbonate (4 to 1) fusion mixture.

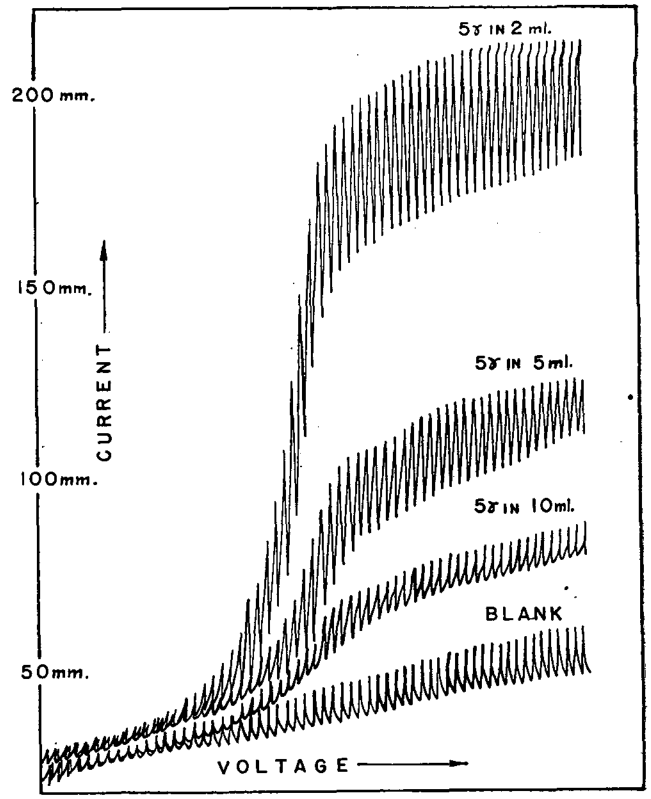

Figure 2. Relative Wave Heights Obtained from 5 Micrograms of Chromium in 2, 5, and 10 Ml. of 1 *N* Sodium Hydroxide Solution

Sensitivity 0.003 μa./mm.

Table III. Polarographic Analyses of Steels

Bureau of Standards Sample No.	Wt. of Sample, Mg.		% Cr Found	% Cr Present[a]
32c	5.44		0.64	
	6.28		0.60	0.654
	6.03		0.68	
		Av.	0.64	
115	4.26		2.07	2.17
	3.66		1.86	2.17
	3.78		2.16	
		Av.	2.03	
121a	1.02		18.3	
	1.05		17.6	18.69
	1.60		19.2	
		Av.	18.4	
152[b]	9.61		0.09	
	9.25		0.11	0.05
	9.78		0.05	
		Av.	0.08	

[a] Certificate values.
[b] Tin bearing.

EFFECT OF SODIUM HYDROXIDE CONCENTRATION

Thanheiser and Willems (*10*) studied the effect of sodium hydroxide normality on the height of the chromium diffusion current wave in 0.5, 1, 2, 4, and 6 *N* sodium hydroxide solutions. It was desirable to know the effect of a slight variation in concentration from the usual 1 *N* sodium hydroxide solution. Figure 3 shows the results obtained with 50 micrograms of chromium in 10.0 ml. of alkaline solution. The general trend agrees well with Thanheiser and Willems' results. It shows that in the vicinity of 1 *N* sodium hydroxide, a slight variation of normality has little or no effect on the wave height. Higher well formed waves are obtainable in 0.1 to 0.4 *N* solutions, but the change in height of the wave with small variations in normality is much greater. It is possible that better waves may be obtained in buffered solutions. However, at low normalities the polarograms of chromate solutions show an additional wave at 0.3 volt *vs.* the saturated calomel electrode. At normalities greater than 0.2 *N* this wave tends to disappear.

INTERFERENCES

The effects of increasing amounts of various substances upon the recovery of 50 micrograms of chromium are shown in Table IV. The chromium and the foreign substance were mixed in 250-ml. beakers, diluted with 150 to 175 ml. of distilled water, and run through the procedure for filtrates. The acids were either dried or fumed for 0.5 hour before diluting with water. Sulfuric and nitric acids have little effect. Phosphoric acid has no effect in small quantities, but in larger quantities it renders the waves unmeasurable. Hydrochloric acid shows a peculiar tendency to cause low results when present in amounts ranging from 10 to 100 mg. When concentrations of 500 to 1000 mg. of hydrochloric acid are used the effects are absent. Check analyses give the same results. Perchloric acid gives low and erratic results at all times. The authors have not been able to use this acid successfully. Chapman *et al.* (*2*) reported similar effects in the treatment of chromium compounds with perchloric and hydrofluoric acid solutions. The sodium salts of the common acids do not seem to interfere. Of the metals studied, lead, zinc, and cadmium interfere the most. The lead wave comes just before the chromium wave, and in the procedure, the lead and chromium waves merge, giving high results for chromium. The zinc, cadmium, and copper curves interfere to the extent that their maxima or high residual currents, due to the comparatively large amounts being used, tend to merge or flow into the chromium wave. Cobalt, iron, manganese, and nickel interfere only to the extent that their bulky precipitates adsorb or entrap the chromium ions. As a rule, if the precipitate is not too bulky, there is no interference.

Table IV. Chromium Recovery

(Micrograms of chromium recovered from 50 micrograms added)

Substances Added	0	1 mg.	10 mg.	50 mg.	100 mg.
HNO_3	48	45	49	47	57
	47	47	47	45	53
H_2SO_4	49	54	49	53	53
	50	50	52	57	47
H_3PO_4	47	48	48	53	1035
	53	47	49	52	552
HCl	51	50	33	39	40
	49	49	32	40	36
$HClO_4$	50	35	45	41	25
	54	35	41	23	37
$NaNO_3$	47	47	50	52	48
	47	50	50	48	49
Na_2SO_4	49	49	51	47	47
	50	48	48	48	49
Na_3PO_4	53	54	53	56	52
	51	57	53	57	55
NaCl	48	47	48	51	52
	49	49	53	48	50
Na_2CO_3	50	52	53	55	50
	49	50	53	58	47
Br_2	53	49	50	49	54
	54	49	51	53	53
Cd (as $CdSO_4$)	48	57	[a]	[a]	[a]
	52	62			
Co [as $Co(NO_3)_2$]	53	51	47	[b]	[b]
	50	50	46		
Cu (as $CuSO_4$)	53	51	51	[a]	[a]
	51	53	48		
Fe [as $Fe(NH_4)_2(SO_4)_2$]	51	50	44	3	[b]
	50	46	58	4	
Mn [as $Mn(NO_3)_2$]	48	45	35	14	[b]
	47	48	35	14	
Mo (as $MoO_3 + NH_4OH$)	52	48	52	43	50
	48	50	48	47	43
Ni (as $NiSO_4$)	53	51	45	2	[b]
	50	53	44	2	
Pb [as $Pb(NO_3)_2$]	54	200	1200	[a]	[a]
	51	200	1150		
Pb (as $PbSO_4$)	..	194	950	2800	[a]
		194	950	2250	
Zn (as $ZnSO_4$)	51	65	72	[a]	[a]
	49	85	102		

[a] Interference due to adsorption, absorption, and/or entrapment.
[b] Interference from maxima or high residual currents.

CRUCIBLES

Practically all the fusions were carried out in platinum crucibles. There is some loss of platinum, but the cost of the platinum lost is small in comparison to the ultimate cost of other types of crucibles. Satisfactory results may be obtained by fusing the chrome ore in porcelain crucibles with sodium peroxide. Care has to be taken to let the melt solidify on the sides of the crucibles, lest the crucibles be cracked. The magnesium oxide–sodium carbonate fusion mixture cannot satisfactorily be used with the porcelain crucibles; the melt vitrifies upon solidifying and becomes very difficult to dissolve. Unsatisfactory results were also obtained upon using nickel crucibles with the magnesium oxide–sodium carbonate fusion mixture; the chromate pitted the sides and bottom of the crucibles and gave low, erratic results. Electroplating the insides of the nickel crucibles with new nickel surfaces did not help. Sodium carbonate–sodium nitrate fusion mixtures worked well for chromium oxides and compounds, but could not satisfactorily fuse chrome ore. Iron crucibles were not tried for small samples because of the possible formation of large amounts of ferric hydroxides.

SUMMARY

A polarographic method for the determination of chromium in dusts and mists is described. It is essentially a micro or semimicromethod and consists of two parts: one for solutions and one for residues. The solutions are oxidized with hydrogen peroxide in alkaline solution, dried, and run polarographically in 10.0 ml. of 1 *N* sodium hydroxide solution. The residues are fused with a 4 to 1 mixture of magnesium oxide and sodium carbonate, soaked with 10.0 ml. of 1 *N* sodium hydroxide solution, centrifuged, and run polarographically. The lower limit of sensitivity is 0.2 microgram of chromium per milliliter of final solution. Theoretical and observed diffusion current values agree within expected limits. Results obtained with the proposed method are compared to those

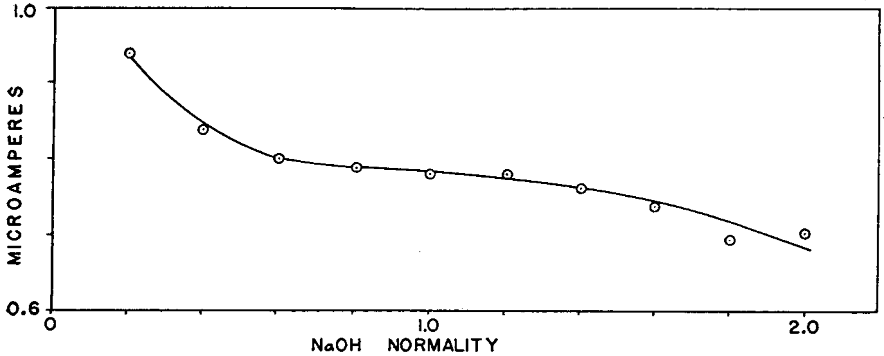

Figure 3. Effect of Sodium Hydroxide Concentration on Diffusion Current

50 micrograms of chromium in 10.0 ml. of alkaline solution

obtained by standard macromethods. A number of National Bureau of Standards samples were also analyzed. Results fall within an average deviation of ±4% of the amount present. A number of factors influencing the accuracy of the determination have been studied and their effects tabulated. More than 1000 routine analyses have been made with the method described.

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Microdetermination of Knock Characteristics of Motor Fuels

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A method is described whereby motor and research octane numbers can be determined on 20-ml. samples of fuel with an accuracy approaching that of the standard knock engine test procedures. This method employs the customary knock engine and involves two alterations in design and one change in procedure: The amount of waste fuel in the fuel inlet system is reduced from 60 to 1 ml. by the use of a specially designed glass capillary inlet system. The running time necessary for the standard knock meter to reach equilibrium is eliminated with negligible loss in accuracy by its replacement with an instantaneous recording knock meter. The running time necessary for the adjustment of the compression ratio and the fuel-air ratio is reduced by the use of several manipulative shortcuts including a continuously variable fuel-air ratio. The standard deviation for 42 research octane determinations on different fuels was 1.42, and that for 38 motor octane determinations was 1.15.

THE standard methods for the determination of the knock characteristics of motor fuels which have been developed during the past decade by the petroleum industry and have recently been standardized by the American Society for Testing Materials (*1*) have proved of considerable value in studying the products of petroleum research and in controlling the manufacture of motor gasoline. The primary need behind the development of these methods was for a plant control test, with the result that very little thought was given to the size of the sample required for an octane determination. Samples of at least 300 ml. are generally considered necessary for a normal determination. During recent years, in which research on motor fuels has expanded considerably, this minimum requirement for each octane determination has placed a considerable strain on the laboratory and pilot plant equipment used to prepare experimental batches of gasoline. It has often led to many unnecessary hours being spent repeating an experiment several times in order to build up a sufficient supply of product for the various engine tests which are needed.

The desire of chemists and engineers to overcome this difficulty has led to the unofficial use of two types of modifications of the standard engine test methods. The first of these has involved blending the unknown sample with a fuel of known octane value, determining the octane number of the blend, and then correcting for the contribution of the diluent by means of empirical conversion tables or charts. The weakness of such procedures lies in the fact that different blending charts must be used for different types of fuels. This leads to uncertain results when unknown samples are being evaluated.

The second type of modification has involved reducing the holdup of sample in the carburetor and float bowl of the test engine by the use of a small-scale commercially available carburetor, or a specially built substitute fuel container. A number of such modifications have been tested by various engine groups (*2*, *3*) and information has been circulated throughout the industry, although no published papers are available, according to the best information of the authors. The use of such modifications as those just described reduces the minimum sample size to 80 to 100 ml.

The Houdry Laboratory has employed one of these modifications for over a year with considerable success, but has felt that for research purposes the fuel requirement was still greater than desired. It therefore undertook the study described in this paper, and has succeeded in developing a method whereby octane num-

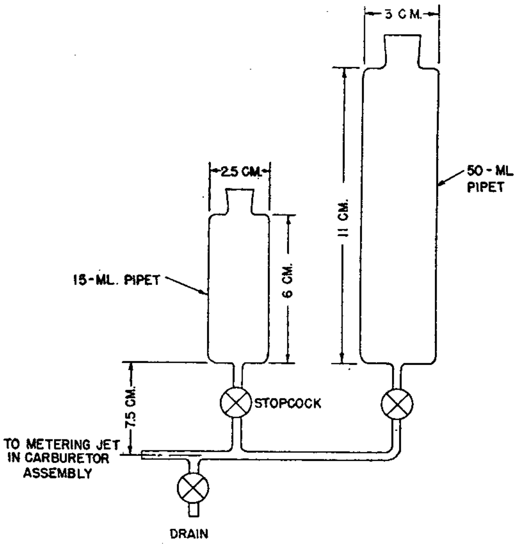

Figure 1. Modified Fuel Inlet System for Octane Microdetermination

ber, accurate to better than ±2 units, can be determined without dilution on samples as small as 20 ml. in size, in the same time required for full scale octane number determinations. The accuracy of this method is not as great as that of A.S.T.M. methods. However, it offers a useful tool in connection with laboratory size investigations.

FACTORS CONTRIBUTING TO USE OF EXCESS FUEL

A careful examination of the standard knock engine equipment and testing procedure has indicated that there are three major factors contributing to the use of excess fuel:

The fuel inlet system on the standard engine, including the float bowl, the carburetor, the fuel level gage, and the connecting lines, has a waste capacity of approximately 65 ml. of fuel which cannot be utilized in the course of the determination.

The response of the standard knock meter is extremely sluggish. It takes approximately 1 minute or 20 ml. of fuel to level out after each change of fuel or adjustment of conditions.

Considerable running time on the test fuel is consumed in adjusting first the compression ratio, and then the fuel-air ratio, in order to give maximum knock.

Each of these factors has been carefully studied, and modifications of the equipment and procedure have been made in each case which reduce fuel consumption to a minimum.

Modified Fuel Inlet System. In order to reduce the holdup of the fuel inlet system to an absolute minimum, the fuel bowl, the fuel level gage, and all the connecting lines leading to one of the carburetor positions of the standard knock engine have been eliminated and replaced by a glass inlet system as indicated in Figure 1.

This inlet system consists of a 50-ml. pipet 3 cm. in diameter, and a 15-ml. pipet approximately 2.5 cm. in diameter, connected with each other and with the entrance to the carburetor valve by capillary tubing. The 15-ml. pipet is located on the carburetor assembly in such a way that it is on the same level as the central part of the standard fuel level gage. By the use of this inlet system the amount of wasted fuel is reduced to approximately 1 ml.

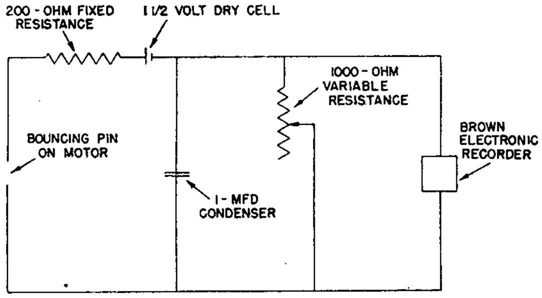

Figure 2. Wiring Diagram of Modified Knock Meter for Octane Microdetermination

Quick Response Recording Knock Meter. It is well understood that the slow response of the standard knock meter is the result of deliberate design, the intention being that the continual variation of knock intensity experienced with even the mildest fuels be ironed out by the lag in the knock meter. The investigation in this laboratory showed that equally good average meter readings could be obtained with an instantaneously reading meter which was allowed to plot a continuous record. The most satisfactory recording meter was found to be the Brown electronic recording potentiometer. This potentiometer is used to measure the fluctuating voltage of a small electrical condenser which is intermittently charged by means of a current flowing through the bouncing pin contacts attached to the motor, and continuously discharged through a fixed resistance. Figure 2 illustrates the electrical circuit used for this purpose.

Changes in Procedure Decreasing Time Requirement for Adjusting Compression Ratio and Fuel-Air Ratio. The trial and error method for determining the proper compression ratio and fuel-air ratio has been replaced by a modified operating procedure which determines these adjustments in a more automatic fashion.

A 15-ml. blend consisting of 5 ml. of sample and 10 ml. of an 80-octane reference fuel is run in the engine at a compression ratio based upon the best available guess as to the octane number of the sample. It is held in the 15-ml. pipet shown in Figure 1 and as fuel is burned the fuel level drops continuously, allowing the fuel-air ratio to pass through its maximum. The curve obtained on the recording knock meter passes through a corresponding maximum value and this reading is used to indicate the approximate octane number of the blend when bracketed between readings on the same knock meter on two reference fuels. The compression ratio of the engine is reset in accordance with the octane number of the sample as estimated from this blend. A second determination is then made on 15 ml. of the sample without dilution, using the same procedure.

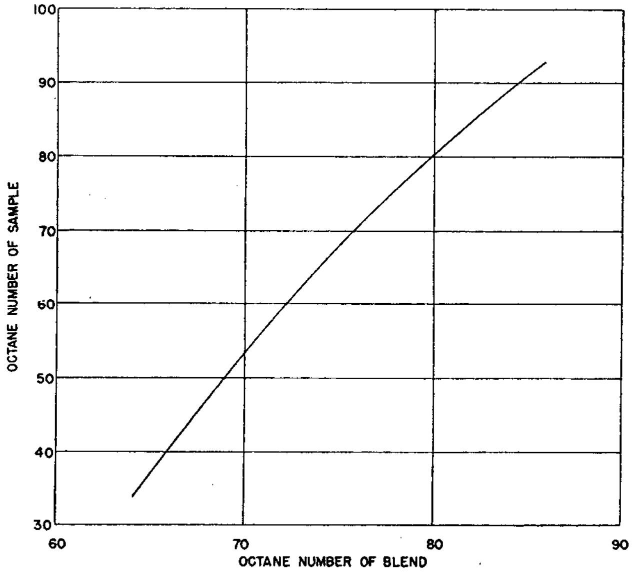

Figure 3. Octane Number of Sample *vs.* Octane Number of Blend

33.3% sample and 66.6% 80 octane reference fuel research test procedure

In practice it has been noted that the knock intensity falls off sharply when switching from one fuel to another by normal means. Therefore, the 50-ml. pipet shown in Figure 1 was added. A fuel with behavior somewhat similar to that of the sample or blend being tested is fed to the engine from this pipet to precondition the engine before introducing the test sample. When its level reaches that of the sample in the 15-ml. pipet, the stopcock at the bottom of the 15-ml. pipet is opened and that at the bottom of the 50-ml. pipet is subsequently closed. This smooth transition from one fuel to another avoids a drop in octane at the time of switching, a drop which seriously affects the shape of the curve registered during the burning of the 15-ml. sample.

INSTALLATION OF EQUIPMENT

The metering jet plug and metering jet of the standard antiknock engine, either motor or research, are removed from the center bowl of the carburetor assembly. These are replaced by a special jet of exactly the same bore as the standard one, 0.031 inch for the research and 0.027 inch for the motor method. This special jet has an extended threaded section 3.25 cm. (1.5 inches) long, to the end of which is silver-soldered a piece of $^1/_8$ inch copper tubing 1.25 inches long. It is installed by simply screwing it into the opening left by the standard metering jet and plug, and is held firmly in position by a lock nut which is tightened up against the case. Thus it completely by-passes the standard 300-ml. carburetor tank and fuel level sight glass. The manifold of the two glass pipes is connected directly to this jet, as indicated in Figure 1. The glass pipets are held in place by clamping them directly to the carburetor assembly. The recording knock meter is installed by removing the leads to the standard knock meter from the bouncing pin and connecting the wiring circuit shown in Figure 2 to the contact terminals.

Table I. Research Method

(A.S.T.M. Designation D 908–47T)

Sample Description	Determination	A.S.T.M. Procedure	Octane Microprocedure	Deviation
Catalytically cracked East Texas gas oil (experimental conditions)	1	87.5	88.0	+0.5
	2	87.5	88.5	+1.0
	3	51.0	49.8	−1.2
	4	83.8	84.0	+0.2
	5	87.8	88.0	+0.2
	6	88.6	87.0	−1.6
	7	92.6	93.3	+0.7
	8	91.5	90.7	−0.8
	9	89.3	88.4	−0.9
	10	91.0	93.2	+2.2
	11	89.0	89.0	0.0
	12	89.4	88.3	−1.1
	13	89.7	88.0	−1.7
	14	90.2	89.8	−0.4
	15	90.6	91.6	+1.6
	16	89.0	88.0	−1.0
	17	91.0	90.8	−0.2
	18	91.2	89.5	−1.7
	19	91.0	89.4	−1.6
	20	91.4	91.2	−0.2
	21	91.2	90.7	−0.5
	22	89.7	89.0	−0.7
	23	90.1	89.2	−0.9
Thermally cracked East Texas gas oil	24	63.0	63.2	+0.2
	25	61.3	64.0	+2.7
	26	61.5	64.0	+2.5
	27	60.0	58.5	−1.5
	28	62.0	64.9	+2.9
	29	60.0	62.5	+2.5
	30	60.0	62.4	+2.4
Thermally cracked 15% East Texas bottoms	31	59.6	59.3	−0.3
	32	64.4	63.6	−0.8
	33	64.2	65.5	+1.3
	34	64.0	62.0	−2.0
	35	60.5	62.8	+2.3
	36	64.8	65.4	+0.6
Thermally cracked 16.1% West Texas bottoms	37	65.2	63.3	−1.9
	38	68.6	69.5	+0.9
	39	65.3	66.5	+1.2
Thermally cracked West Kansas crude	40	58.3	57.0	+1.3
	41	61.0	61.3	+0.3
		60.0	60.0	0.0
	42	Standard deviation (σ)		1.42

PROCEDURE

The procedure requires a sample of 20 ml. and an estimated octane value that should be accurate to within 20 octane numbers. This value should be as accurate as possible, as closer estimates usually give more reliable results.

Divide the unknown sample into two parts—a 5-ml. and a 15-ml. fraction. Blend the 5-ml. fraction with 10 ml. of 80-octane reference fuel.

Determine the approximate octane rating of this blend from either Figure 3 or 4, using the estimated octane number of the sample.

Set the compression ratio of the engine to agree with the estimated octane number of the blend.

Place the blend in the 15-ml. pipet and a reference fuel of the same estimated octane number in the 50-ml. pipet and also in one of the standard 300-ml. carburetor tanks.

Turn on the recording knock meter and turn the reference fuel in the standard carburetor tank into the motor. Adjust the variable resistance in the circuit until this reference fuel makes a reading slightly above the center of the chart.

When the chart has been correctly adjusted, switch the motor from the fuel in the 300-ml. tank to that in the 50-ml. pipet. As the liquid level in the pipet falls, the correct air-fuel ratio is approached and the reading on the knock meter rises. The pipets are so constructed that when the level in the 50-ml. pipet falls to the level in the 15-ml. pipet the reading on the chart is about halfway to its peak. At this point open the stopcock to the 15-ml. pipet and subsequently close off the 50-ml. pipet. Allow the 15-ml. pipet to drain completely.

Bracket the maximum reading made by the blend with readings of two reference fuels on the same knock meter, using the standard carburetors. Allow these fuels to run long enough to get good average readings. Read the curve made by the blend by taking the average of the points on the peak of the curve.

Determine the octane number of the blend from the above three values in the same manner, as would be done when using readings from a standard knock meter. From the octane value of the blend, determine the approximate rating of the sample using either Figure 3 or 4.

Set the compression ratio of the motor again, this time to check with the approximate octane rating of the sample as obtained above.

Fill the small pipet with the 15 ml. of pure sample. Fill the 50-ml. pipet with a reference fuel of approximately the same octane number. However, if the sample is known to have very poor ignition qualities or a poor response, use a gasoline that has the same burning qualities in the 50-ml. pipet instead of a reference fuel.

Table II. Motor Method

(A.S.T.M. Designation D 357–47)

Sample Description	Determination	A.S.T.M. Procedure	Octane Microprocedure	Deviation
A.S.T.M. exchange sample, commercial gasoline	1	69.2	67.2	−2.0
	2	80.4	80.3	−0.1
East Texas gas oil catalytically cracked under experimental conditions	3	77.2	76.9	−0.3
	4	77.0	77.8	+0.8
	5	75.2	74.9	−0.3
	6	76.0	76.9	+0.9
	7	76.3	76.0	−0.3
	8	76.3	76.2	−0.1
	9	72.0	74.0	+2.0
A.S.T.M. exchange sample + 10% *n*-heptane	10	74.3	74.4	+0.1
A.S.T.M. exchange sample + 20% *n*-heptane	11	69.6	67.3	−2.3
A.S.T.M. exchange sample + 40% *n*-heptane	12	54.0	56.4	+2.4
A.S.T.M. exchange sample + 50% iso-octane	13	86.8	84.5	−2.3
Catalytically cracked East Texas gas oil	14	79.6	80.4	+0.8
	15	80.4	79.2	−1.2
	16	80.4	80.0	−0.4
	17	80.4	80.9	+0.5
	18	80.4	79.6	−0.8
	19	80.4	79.2	−1.2
	20	80.7	79.7	−1.0
	21	80.8	81.8	+1.8
	22	80.0	79.5	−0.5
	23	79.6	78.8	−0.8
	24	79.6	79.4	−0.2
	25	80.4	80.0	−0.4
Thermally cracked 15% East Texas bottoms	26	61.2	62.4	+1.2
	27	62.7	61.6	−1.1
	28	62.0	61.3	−0.7
	29	60.0	58.3	−1.7
	30	58.2	59.1	+0.9
	31	58.2	57.6	−0.6
	32	61.0	60.0	−1.0
	33	59.2	60.0	+0.8
	34	59.2	58.7	−0.5
	35	58.2	57.6	−0.6
	36	58.2	57.9	−0.3
115–160° cut from thermally cracked East Texas bottoms	37	70.2	72.0	+1.8
	38	79.6	79.5	−0.1
		Standard deviation (σ)		1.15

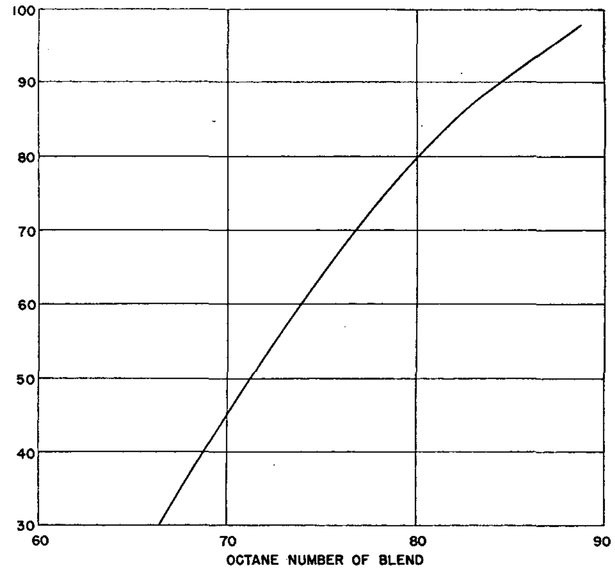

Figure 4. Octane Number of Sample *vs.* Octane Number of Blend

33.3% Sample and 66.6% 80 octane reference fuel Motor test procedure

Fill one of the 300-ml. carburetor tanks with the same fuel that is placed in the 50-ml. pipet and again adjust the recording knock meter so that this fuel will give a reading slightly above the center of the chart.

Turn the fuel in the 50-ml. pipet into the motor. When the fuel level has fallen to the same height as the fuel level in the 15-ml. pipet, open the stopcock to the 15-ml. pipet and close off the 50-ml. pipet. Allow the 15-ml. pipet to empty completely.

Using the regular 300-ml. carburetor tanks, bracket the reading made by the sample with two reference fuels. Calculate the octane rating of the sample exactly as the value for the blend was determined.

Example. Figure 5 illustrates the microdetermination of the octane rating of the research test procedure of a 20-ml. sample of catalytic gasoline.

The estimated octane number of the sample was 92. Using this number and Figure 3, the approximate octane rating of the blend was found to be 85.7. The compression ratio of the motor was set for this value and the blend run as illustrated by points 1 through 5 on Figure 5.

The octane rating of the blend was calculated from the chart readings as follows:

$$\frac{50\%\ \text{iso-octane} - \text{sample}}{50\%\ \text{iso-octane} - 60\%\ \text{iso-octane}} \times 10 =$$

$$\frac{780 - 640}{780 - 560} \times 10 = 6.4$$

$$50 + 6.4 = 56.4\%\ \text{iso-octane}$$

56.4% iso-octane = 82.6 octane number, actual rating of the blend.

From 82.6, the actual octane number of the blend, the first approximation of the value for the sample was obtained as 86. The compression ratio was set for this value and the pure sample run as illustrated by points 6 through 11 on Figure 5.

By coincidence, in this case, 60% iso-octane reference fuel gave exactly the same reading as the sample, 780. Thus the octane rating of the sample was taken as equal to this, or 84.0. The octane number of the sample as later determined by the standard A.S.T.M. research test procedure was 83.8.

BLENDING CURVES

Figures 3 and 4 are plots of the octane numbers of various gasolines versus the octane numbers of blends of one third these same

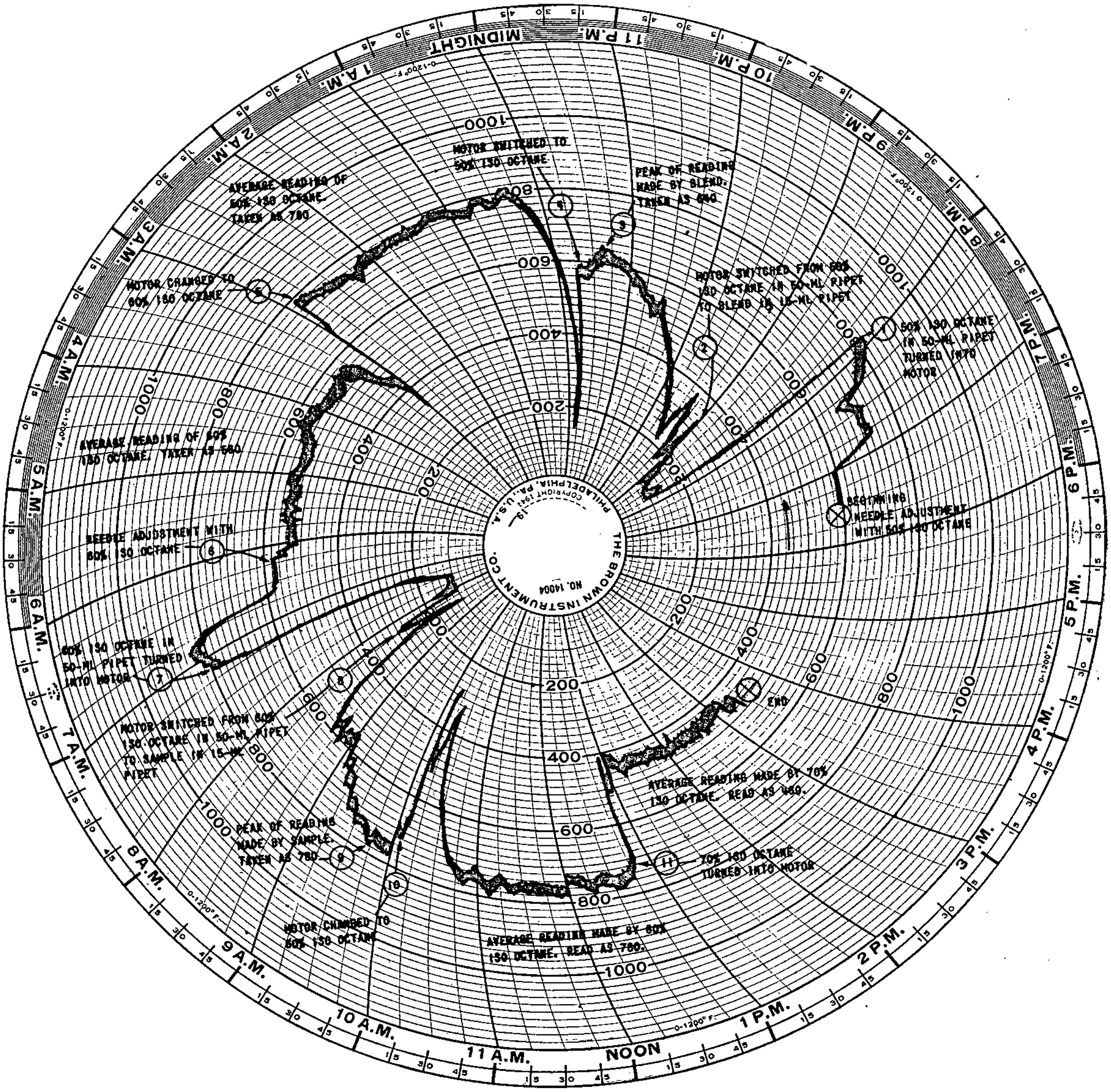

Figure 5. Research Test Procedure, Run 4

Actual chart speed, 1 revolution in 30 minutes. Each hour division, 1.25 minutes

gasolines and two thirds 80-octane reference fuel, as determined by the research and motor test procedures, respectively (A.S.T.M. Designations D 908-47T and D 357-47). It is well known that different types of gasoline will give different blending values. For this reason plots 3 and 4 are actually curves of average values. The curves were drawn through the experimental values of all points determined from a great variety of blends. The stocks actually blended to obtain these curves were catalytically cracked gasolines, thermally cracked gasolines, various reference fuels, A.S.T.M. exchange samples, and several synthetic fuels.

REPRODUCIBILITY OF RESULTS

The method described above has been tested on 38 samples using the motor method and on 42 samples using the research method. The results of these tests are given in Tables I and II. Absolute values for the octane numbers of these samples are, of course, unobtainable. However, a statistical analysis of the deviation in each case between the octane numbers as determined by the microprocedure and by the standard A.S.T.M. procedures indicates the standard deviations for the two methods to be 1.42 and 1.15 octane numbers, for research and motor methods, respectively. The significance of these standard deviations can be ascertained from any probability table, but briefly, they indicate that according to statistical distribution, 69% of all determinations by the micromethod will vary from the corresponding A.S.T.M. determination by no more than the standard deviation (σ). Likewise, 95% of all determinations will be within 2σ of the A.S.T.M. value and 99.7% will be within 3σ.

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Microdetermination of Iodine Employing Radioactive Iodine

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A method is described for the determination of small amounts of iodine in which the quantifying procedure is a radioactivity measurement. Labeled iodine is produced by the reaction of the unknown in the form of iodate with an excess of radioactive iodide of known specific activity. The iodine is extracted with carbon tetrachloride and the radioactivity in the carbon tetrachloride is determined by gamma-ray counting.

SEVERAL methods of considerable sensitivity and accuracy are available for the measurement of small amounts of iodine. Those most commonly used depend either upon the production of a blue color by elemental iodine in the presence of starch, or upon the catalytic action of iodide ion on the reduction of ceric to cerous ion by arsenious acid (*4*). For routine use in the measurement of small amounts of iodine, such as occur, for example, in plasma, it would seem desirable to have a method more sensitive than the starch-iodine method, and less painstaking than the ceric ion reduction procedure.

In the method described, the iodine determination is based upon a radioactivity measurement. As in the starch-iodine method as generally employed, the iodine to be determined is oxidized to iodate, and the iodate is allowed to react with an excess of iodide to form elemental iodine. The iodide solution is labeled with radioactive iodide (I^{131}), and the specific activity of this solution is determined by γ-ray counting of a liquid sample. The iodine formed in the reaction is extracted with carbon tetrachloride and the radioactivity is determined in identical manner.

The essential reaction in the method presented is:

$$I^{127}O_3^- + 5\,I^{-127.131} \longrightarrow 3I_2^{127.131}$$

At the instant of formation, the specific activity of the $I_2^{127.131}$ will be $^5/_6$ that of the $I^{-127.131}$. However, exchange reactions between iodine and iodide occur rapidly, and the specific activity of the iodine will approximate that of the iodide by exchange with the excess of iodide present. Exchange reactions between iodate and iodine or iodide do not occur (*1, 3*).

The amount of labeled iodide used depended upon the general range of the quantity of iodate to which it was added. Although some idea of the range of an unknown iodine was necessary for the determination, the value had only to be estimated within fairly wide limits. In the experiments summarized in Table II, a single amount of iodide proved satisfactory for an 18-fold difference in the amount of iodate. The molar iodide-iodate ratios ranged from 28:1 to 500:1.

Table I. Distribution of Iodide Ion between Water and Carbon Tetrachloride

	Radioactivity, H_2O-CCl_4
First extraction	99.7:0.3
Aqueous phase re-extracted with CCl_4	~100:0
Additional 0.5 ml. of concd. H_2SO_4 added to aqueous phase and re-extracted with CCl_4	~100:0

Radioactively labeled iodide, 20 γ/ml., as potassium iodide, was acidified with 1 drop of concentrated sulfuric acid and extracted with an equal volume of carbon tetrachloride. The radioactivity was determined in each phase.

METHOD

Standard reagents were used. The water was prepared by routine distillation. The labeled iodide solution was prepared each day by adding carrier-free I^{131} to a measured amount of a freshly prepared solution of potassium iodide.

The iodine to be determined, in the form of iodide, was oxidized to iodate by one of two standard methods: (1) the addition to an acid solution of an excess of bromine, followed by boiling to remove the remaining bromine; (2) Gróak's permanganate method (*2*).

In the experiments performed, the sample, in the form of iodate in 2 to 3 ml. of aqueous solution contained in a 50-ml. Erlenmeyer flask, was chilled in an ice water bath. Radioactively labeled

Table II. Determination of Iodate

	Test Sample (I as Iodate Added)	Labeled Iodide		Total Radioactivity, in CCl_4 (after Subtracting Blank)	I as Iodate, Calcd.
		Amt. used in reaction	Specific activity		
	γ	γ	*Counts/sec./γ*	*Counts/sec.*	γ
I	1.8[a]	50	91.4	780.8	1.71
	1.8	50	91.4	785.2	1.72
	0.6	50	91.4	272.4	0.598
	0.6	50	91.4	254.8	0.558
	0.3	50	91.4	116.4	0.256
	0.3	50	91.4	118.8	0.26
II	0.6	50	51.4	177.6	0.69
	0.6	50	51.4	168.8	0.66
	0.6	50	51.4	162.0	0.63
	0.1	50	51.4	31.6	0.12
	0.1	50	51.4	28.4	0.11

A known amount of potassium iodate was allowed to react with labeled iodide. The reaction, carbon tetrachloride extraction, and radioactivity determinations were carried out as described in the text.

[a] Sample Calculation.

$\frac{780.8}{91.4} \times 1/6 \times 6/5 = 1.71$, where 780.8 = counts/sec. in total volume of carbon tetrachloride; 91.4 = counts/sec./γ; $^1/_6$ is factor obtained from $IO_3^- + 5I^- \longrightarrow 3I_2$; and 6/5 is correction for carbon tetrachloride extraction efficiency. The correction for the extraction efficiency (6/5) is a factor which gives an average recovery very close to 100% (100.8%) for all the groups in the table.

For simplicity in calculation, no correction for the small effect of the test sample iodine on the final specific activity has been applied (3.6% for 1.8 γ samples to 0.2% for 0.1γ samples).

iodide in a volume of 0.5 ml. was added and the flask was swirled for mixing. It was not necessary to measure the quantity of added iodide with precision. One drop of concentrated sulfuric acid was added and, after mixing, the reaction was allowed to proceed in ice water for 3 minutes. The solution was then transferred to a graduated centrifuge tube, using additional water to rinse the flask, and the volume was brought to 5 ml. Five milliliters of carbon tetrachloride were added, and the tube was stoppered and shaken for 3 minutes. After centrifuging for 30 seconds, the aqueous phase was completely removed with a rubber bulb pipet. Loss of carbon tetrachloride was not important but to ensure complete removal of the aqueous phase, the sides of the tube were rinsed with several milliliters of water which, too, were completely drawn off. One milliliter of the carbon tetrachloride was then transferred to a standardized vial for radioactive measurement by γ-ray counting.

A distilled water blank was processed with each group of test samples, using water from the same source and the same labeled iodide solution for all samples in the group. The radioactivity count in the carbon tetrachloride extract of the blank was subtracted from the count obtained in each of the other samples, and thus served as a control for any spontaneous oxidation of iodide to iodine.

It was found advisable to have the blank simulate the test solutions as closely as possible, including the addition of equal amounts of all electrolytes used in the test samples. In this way, an automatic correction was made for the presence of any small amount of contaminating iodine in the chemicals used, or for any substance capable of oxidizing iodide to iodine.

As a safeguard against false results due to contamination by reducing substances, it was considered advisable to process a sample containing a known amount of iodine in the general range of the unknowns.

The radioactivity measurements in this work were made in the following manner:

> Liquid samples of 1.0 ml. were counted in standardized 10-ml. flat-bottomed vials. The base of the vial was placed in contact with a shielded and firmly fixed Geiger-Müller tube through a hole in the lead shield cut exactly to size for the standard vial. The Geiger-Müller tube was a brass-encased γ-counting tube of high efficiency (Sylvania, Type GG 306). In this constant geometrical arrangement, 1 microcurie of I^{131} in 1.0 ml. of water or carbon tetrachloride produced 270 counts per second when counted with one tube of this type, and 320 counts per second with another.
>
> The iodide solution was labeled with that amount of I^{131} that would deliver about 10 microcuries to each test sample. The number of atoms contributed by the addition of I^{131} was insignificant and was disregarded. One milliliter of the iodide solution, containing a known concentration of iodide, was diluted fifty times, and 1.0 ml. of the dilute solution was counted in the manner described. The specific activity (counts per second per microgram) was thus determined. In the final step of the procedure, 1.0 ml. of carbon tetrachloride was counted in identical manner.

A sample calculation is presented with Table II.

RESULTS

The satisfactory use of carbon tetrachloride depends upon the ability of this substance to remove iodine selectively from an iodide-iodine solution. It is likely that other solvents with this property will also prove satisfactory. Benzene, for example, appears to be a possible substitute. Table I demonstrates that little or no iodide is extracted from acid water by carbon tetrachloride.

If it may be assumed that the exchange reaction between iodide and iodine goes very nearly to completion during the procedure, then the method itself, applied to known amounts of iodate, becomes the best experiment for determining the efficiency of the carbon tetrachloride extraction of iodine under the conditions of the method. The author's observations suggest that the exchange between iodide and iodine proceeds rapidly. When carrier-free I^{131}, as iodide in water, was shaken for 3 minutes with carbon tetrachloride containing iodine, the distribution of radioactivity between the water and carbon tetrachloride was consistent with that of iodine. If the carbon tetrachloride was then reshaken with water containing iodide equal in amount to the iodine in the carbon tetrachloride, the distribution of radioactivity between water and carbon tetrachloride was consistent with the assumption that the iodide and iodine had attained the same specific activity.

From the results obtained when the method was applied to measured amounts of iodate, it would appear that for the range studied (0.1 to 1.8 micrograms of iodine as iodate), and assuming complete exchange between iodide and iodine, about 83% of the iodine was removed by a single extraction with an equal volume of carbon tetrachloride under the conditions of the experiment. Table II shows the results obtained when calculated on that basis.

The results obtained when measured amounts of potassium iodide were oxidized to iodate with bromine and then determined by the method outlined are presented in Table III.

Table III. Determination of Iodide

Test Sample, Iodide Added	Labeled Iodide		Total Radioactivity in CCl_4 (after Subtracting Blank)	Iodide Calcd.
	Amt. used in reaction	Specific activity		
γ	γ	*Counts/sec./γ*	*Counts/sec.*	γ
5.0	500	10.63	252.5	4.76
2.0	200	10.63	99.6	1.87
1.0	200	10.63	57.6	1.08
0.4	100	10.63	19.8	0.372

Iodide, as potassium iodide, was oxidized to iodate with bromine. After excess bromine was expelled by gentle boiling, procedure and calculations were performed as in Table II.

The presence of any agent capable of reducing iodine will invalidate the results. It appears advisable, therefore, to process a sample containing a known amount of iodine with each group of unknowns. A potential hazard in this regard is the weak sodium bisulfite solution in which I^{131} is bottled at Oak Ridge. Using diluted solutions of the I^{131} as initially received, which have been standing in the laboratory variable lengths of time, no difficulty has been encountered, although the addition of relatively large amounts of sodium bisulfite to the reaction interfered markedly. As sulfite is easily oxidized by aeration, it is anticipated that no difficulty will be encountered in handling this problem should it arise.

The following procedure was carried out to test for the actual presence of sodium bisulfite in the I^{131} solution as it arrived from Oak Ridge.

The sample tested was stated to contain I^{131} in a 0.045 *N* basic solution of sodium bisulfite. The 0.1-ml. aliquots of the solution which were examined would therefore have contained 468 micrograms of sodium bisulfite. The aliquot was diluted to 5.0 ml. and, after the addition of 1 drop of sulfuric acid, was shaken immediately with an equal volume of carbon tetrachloride containing in one instance 5.0 micrograms and in another 20 of iodine. The distribution of the radioactivity between the water and carbon tetrachloride was determined, and the radioactivity found to be very largely in the carbon tetrachloride in both cases. However, when 50 micrograms of sodium bisulfite from a freshly prepared solution were added and the same water and carbon tetrachloride were reshaken, the radioactivity was found to be almost entirely in the aqueous phase. A 0.1-ml. aliquot thus failed to contain sufficient sodium bisulfite to reduce as little as 5.0 micrograms of iodine.

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RECEIVED July 14, 1949.

Round-Table Discussions

One of the special features of the program of the Division of Analytical Chemistry at the national meeting of the A.C.S. in Atlantic City last September was the inauguration of round-table discussions. Realizing the potential interest and value in this type of meeting the division's officers arranged for three discussion groups. The subjects covered were Polarographic Behavior of Organic Compounds, The Karl Fischer Reagent for Use in Determination of Water, and Determination of Carbon in Ferrous Alloys. All three sessions were well attended. The splendid work of the moderators and their panels and the active participation of the audience in each case resulted in stimulating sessions. *Analytical Chemistry* obtained complete stenographic reports of the discussions and turned them over to the moderators for condensation and rewriting. We are happy to publish the resulting reports on the following several pages. We also take this occasion to express the hope that this type of round-table sessions with lively discussion from the floor will be continued and expanded at divisional meetings.—The Editors

Polarographic Behavior of Organic Compounds

Moderator: **PHILIP J. ELVING**, *The Pennsylvania State College, State College, Pa.*

Panel: **OTTO H. MÜLLER**, *Syracuse University, Syracuse, N. Y.*

STANLEY WAWZONEK, *Iowa State University, Iowa City, Iowa*

MELVIN J. ASTLE, *Case Institute of Technology, Cleveland, Ohio*

LOUIS MEITES, *Yale University, New Haven, Conn.*

THE round-table discussion centered around the methodology for studying the behavior of organic compounds by polarographic measurements. The role of various factors and techniques was considered in the light of recent developments in polarography. The principal topics considered included: (1) the effect of environmental factors such as ionic strength, buffer capacity, specific buffer components, maxima suppressors, and pH; (2) criteria for reversibility; (3) the deduction of electrode reaction mechanisms from the variation of half-wave potentials with pH, and the value of *n*, the number of faradays of electricity involved in the reaction at the electrode of 1 mole of the active species; (4) factors determining reducibility or reactivity at the dropping mercury electrode; and (5) current-controlling processes.

ROLE OF THE ENVIRONMENT

In his discussion of the role of the environment, Müller pointed out that the usual excess of indifferent electrolyte ensures a high ionic strength in the solution and that the so-called excitation phenomena are not too well described and have not been too important in practical analysis. In discussing buffer systems, difficulties were described which may arise when the rate of equilibrium attainment in the buffering system is slow compared to the limited time spans involved in using the dropping mercury electrode; specifically, the failure was pointed out of bicarbonate buffers to react fast enough to maintain pH constant at the mercury-solution interface. In unbuffered solutions, quinhydrone gives two separate waves, because the pH at the electrode varies from 2 to 10 as a result of the electrolytic processes which consume or liberate hydrogen ions. In the presence of a buffer of low capacity the curve obtained is something in between those obtained in buffered and unbuffered solutions; actually, the curve represents an acid-base titration at the electrode face. The need for more buffers in the alkaline region was mentioned.

Discussion brought out the general nature of the phenomenon occurring in solutions of poor buffering capacity—i.e., the interchange of hydrogen ions between the constituents of the buffer and the solution seems to be generally rather slow. Even with perfectly adequate buffers, the slowness of dissociation and association of the reactants and products may be the cause of the appearance of several waves if the reactant that is being reduced is involved in the slow process.

It was emphasized that buffers that are suitable for use with the dropping mercury electrode may be ineffective because of inability to reach equilibrium in time when used with electrodes in which the reactions have to go on much faster. Such electrodes include the rotating platinum electrode, the by-pass electrode in which the solution streams past a microelectrode, and the systems used in cathode-ray oscillographic polarography. The inability of the buffer to maintain the pH constant at such electrodes may be due to rate phenomena rather than to buffering capacity.

The effect of the environment, Astle emphasized, may account for the lack of agreement between different investigators on polarographic data, particularly concerning the half-wave potentials of compounds reduced at the dropping mercury electrode. The latter potentials will vary with the nature of the buffer constituents, even though the pH is kept constant. Addition of small amounts of an organic solvent such as alcohol may result in profound change in the reduction potential of a compound.

The substituted benzaldehydes give two waves in a buffer containing glycine but only one wave in buffers of the same pH containing phthalate or citrate. Thus possibility of interaction of the buffer ions with the reactive system should be considered. In the reduction of methylene blue in the redox system, methylene leuco-methylene blue, in a phosphate buffer, the lower part of the curve obtained in an acetate buffer is missing; this is apparently due to the formation of an insoluble product of the phosphate with the leuco form of the methylene blue.

It was pointed out that the type of minimum which had been reported in the polarographic wave of phenolphthalein could also be observed in substituted aromatic nitro compounds in glycine buffers (but not in citrate buffer), in certain inorganic compounds, and in almost any system by the addition of sufficient surface-active material.

Discussion emphasized that a buffer would provide adequate capacity only if used at its pK value where pK represents the negative logarithm of the ionization constant of the active component of the buffer system—that is, the effective buffering range of a buffer system is the pH range of (pK − 1) to (pK + 1).

POLAROGRAPHIC REVERSIBILITY

In beginning the discussion of the criteria for reversibility in polarographic phenomena, Müller discussed the meaning of reversibility. The electrochemist studying nonpolarizable or indicator electrodes in oxidation-reduction systems considers a system reversible if the same potentials are obtained, whether the oxidized form is reduced to known amounts of the two forms or the reduced form is oxidized to the same ratio of the two forms; if a constant and reproducible value is obtained for the redox potential in a 50:50 mixture of oxidized and reduced forms, the system is considered reversible. The situation in polarography is confused, inasmuch as the end products of polarographic reductions are rarely polarographically oxidizable. The use of oscillographic techniques in presenting simultaneously both oxidation and reduction processes at the mercury electrode seems advantageous in evaluating reversibility, although oscillographically observed reversibility and irreversibility may not be identical to the ordinarily observed polarographic phenomena or to potentiometric measurement. It must be kept in mind that irreversible and reversible steps may accompany or follow each other. Thus, the reduction of oxygen to hydrogen peroxide is reversible, while the subsequent conversion of hydrogen peroxide to hydroxyl ion or water is irreversible.

REACTION MECHANISMS

The discussion of the question of what actually occurs at the dropping mercury electrode was opened by Wawzonek, who summarized the present status of the experimental approach to the subject and the usual theoretical interpretation.

Reaction mechanisms at the dropping mercury electrode are best determined by isolating the products formed. In cases where such a procedure is not carried out, the factors important in deducing a reaction mechanism at the dropping mercury electrode in their order of importance are: (1) the determination of *n* by coulometric measurements, from the Ilkovič equation or from a comparison with the polarographic behavior of a compound of similar size; (2) the chemical properties of the compounds concerned; (3) the variation of half-wave potentials with pH; and (4) the slope of the wave.

The determination of *n* is carried out most exactly by coulometric measurements during a reduction at controlled potentials and is the most accurate method when the number of electrons involved is large, as with picric acid. In simple cases involving only a few electrons per unit, *n* can be determined by substituting estimated values of the diffusion coefficient in the Ilkovič equation. The value of *n* will give an indication of the final product formed at the electrode. Thus, if *n* is 4 in the reduction of nitrobenzene, the product formed must be phenylhydroxylamine.

The chemical properties of the compounds concerned are important. The products postulated as being formed at the dropping mercury electrode should not be reducible further at the electrode or should not react with the electrolyte used. An example in which this point may be important is the reduction of picric acid in 0.1 *N* hydrochloric acid. Coulometric measurements indicate a 17-electron change. Reduction to triaminophenol requires 18 electrons. To explain these facts, it has been suggested that the reduction proceeds to the substituted hydrazobenzene. A question can be raised about the stability of this product toward 0.1 *N* hydrochloric acid, since similar conditions bring about the benzidine rearrangement with hydrazobenzene itself. The benzidine if produced would be stable to further reduction at the drop, while the hydrazobenzene represents a compound which can be reduced further, at least chemically, to an amine.

The shift of half-wave potentials to more negative values with an increase in pH can be explained by either a direct reaction of the compound with acid to form an intermediate ion which is more easily absorbed or reduced,

$$\mathrm{R_2C{=}O + H^+ \rightleftharpoons R_2\overset{+}{C}{-}OH}$$

$$\mathrm{R_2\overset{+}{C}{-}OH} + e \longrightarrow \mathrm{R_2\underset{\circ}{C}{-}OH}$$

or by a reaction of the molecule directly followed by a reaction of the anion with water in the following manner:

$$\mathrm{R_2C{=}O} + e \longrightarrow \mathrm{R_2\underset{\circ}{C}{-}O^-}$$

$$\mathrm{R_2\underset{\circ}{C}{-}O^- + H_2O \rightleftharpoons R_2\underset{\circ}{C}{-}OH + OH^-}$$

In both cases the intermediate radical may dimerize or be reduced further to the carbinol. The speed of the reaction of anion with water would determine whether the curve would be dependent upon pH or not.

The slope of the wave gives an indication of the potential-determining step. This value turns out to be usually around 0.06 or higher. The higher values are usually considered as evidence of the irreversibility of the reaction. A possible mechanism of reduction, suggested by Pearson for nitro compounds which may be more general and which would give such values for the slope of an irreversible system involving hydrogen ions, is the following:

$$\mathrm{H^+} + e \rightleftharpoons \mathrm{H(Hg)} \quad \text{(overvoltage)}$$

$$\mathrm{A} + n\mathrm{H} \longrightarrow \mathrm{AH}_n \quad \text{(in alkaline or neutral solution)}$$

$$\mathrm{A} + n\mathrm{H} + \mathrm{H^+} \longrightarrow \mathrm{AH}^+_{n+1} \quad \text{(in acid solutions)}$$

The potentials for such systems would be dependent upon the diffusion coefficient of the reducible material and upon its reaction rate with hydrogen atoms.

The difficulty of answering questions concerning reversibility and reaction mechanisms in polarographic studies was stressed by Meites, who used examples of the behavior of inorganic substances to develop his discussion. Thus, surface-active materials of all types will, if present in sufficiently high concentration, shift the half-wave potentials of polarographic systems, the magnitude of the shift varying with the concentration of the surface-active material—e.g., the addition of sufficient gelatin to an alkaline tartrate solution (pH 14) of copper shifts the half-wave potential of the copper by 1.3 volts while the wave still retains its reversible character. The effect of surface-active materials in producing two-step waves was also considered. A theory was outlined which attempts to explain certain observed changes in half-wave potential and diffusion current on the basis of the formation of highly hydrated micelles whose concentration can be correlated with the addition of surface-active agent and the vapor pressure of water above the solution. The rate of micelle formation and difference in rate of diffusion of micelles of varying degree of hydration may account for some types of two-step waves. It was suggested that rather than to try to suppress maxima by the addition of a substance such as gelatin, which might result in unpredictable changes in polarographic behavior of the substance under study, it would be preferable to remove the maxima by increasing the dilution so that the concentration of the active species was not more than a few hundredths millimolar.

It was brought out in subsequent discussion that readily interpretable waves are very difficult to obtain for organic compounds when present in concentrations below 0.1 to 0.5 millimolar. It was also indicated that pH will alter the effect of varying the concentration of gelatin in a solution. Arguments were advanced for and against the explanation of the effect of gelatin as being due to adsorption of the gelatin on the surface of the drop.

In a discussion of the coulometric measurement of *n* values, microcoulometric cells using dropping mercury were considered as a possible way of evading the assumption usually made that the behavior at a large stirred surface is comparable to that which occurs at the mercury drop. It was pointed out that one could not always depend on stirring by the falling mercury in the microcoulometric cell to avoid local depletion of the solution.

Among those taking extensive part in the discussions were J. K. Taylor, National Bureau of Standards; G. A. Crowe, Hercules Powder Company; Louis Lykken, Shell Development Company; W. W. Davis, Eli Lilly & Company; K. L. Metcalf, E. I. du Pont de Nemours & Company; and P. A. Geary, Smith, Kline & French Laboratories.

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Karl Fischer Reagent for Use in the Determination of Water

Moderator: JOHN MITCHELL, JR., *E. I. du Pont de Nemours & Company, Inc., Polychemicals Department—Chemicals, Wilmington, Del.*

Panel: L. R. KANGAS, *Hercules Powder Company, Wilmington, Del.*

WILLIAM SEAMAN, *Calco Chemical Division, American Cyanamid Company, Bound Brook, N. J.*

THE Karl Fischer reagent is a solution of iodine, sulfur dioxide, and pyridine in methanol. Each of these components enters into the basic two-step reaction for water. Normally, sulfur dioxide, pyridine, and methanol are present in excess. Therefore, the strength of any preparation is dependent on the iodine concentration.

For general laboratory use the reagent may be prepared to contain the components in the molar ratios, $1I_2:3SO_2:10C_5H_5N$ in methanol, at a concentration equivalent to 3 to 4 mg. of water per ml. of reagent. This composition gives a reagent suitable for the titration of samples of extremely low to high water concentrations, minimizes some types of interference, is a good general solvent, does not degrade to darkly colored end products, and assures an excess of components other than iodine. However, for specialized purposes other ratios may be better suited—for example, a reagent containing a higher concentration of pyridine (*20*) is better suited for the determination of water in acetone. Of course, the same effect can be obtained by employing pyridine as an inert diluent for the sample. A preparation containing less pyridine, such as 4 to 5 moles per mole of iodine, is satisfactory for analyses for water in alcohol. A reagent of about one third the water equivalence is useful for titrations of samples containing trace quantities of water (*8*).

Contrary to some reports, the Fischer reagent may be employed for the titration of samples containing amounts varying from minute traces of moisture to pure water. Obviously, as the concentration of water becomes high more care must be exercised in weighing.

Normally, titrations are made by delivering the complete reagent from a desiccant-protected buret into the sample to be analyzed. Because the reagent is subject to parasitic side reactions, which effect a gradual reduction in strength, daily standardizations are necessary. These can be made by titrations of a methanol or ethanol solution containing a known quantity of water, water-saturated alcohols, a weighed amount of water, or a stable hydrate. Incidentally, the reagents prepared to be equivalent to only 1 to 2 mg. of water per ml. are more stable than preparations of higher iodine concentrations (*8*). Thus, Wiberley reported that a reagent equivalent to 1 mg. of water per milliliter was stable. By employing desiccant tubes packed with phosphorus pentoxide on asbestos, he found that usually only weekly standardizations were necessary (*21*). Reagent left in the buret appears to degrade more rapidly than that in the reservoir, possibly because of the increased exposure to light (*18*). Fischer reagent is an extremely active desiccant. Adequate protection against exposure to atmospheric moisture must be maintained for both the stored reagent and the reagent being delivered from a buret into the sample to be analyzed. In areas of high humidity titration in a closed system might be beneficial. A rubber sheet or polythene film can be used to cover the titration flask. Adequate protection is maintained by inserting the buret tip through a hole in the sheet (*6*, *16*).

A modification of the titrimetric technique was suggested by Johannson (*3*), who proposed a divided reagent. A solution of pyridine and sulfur dioxide in methanol was added to the sample flask, and a second solution of iodine in methanol was delivered from a buret. On contact with the contents of the sample flask active Fischer reagent was formed which reacted immediately with the water in the sample. In this way a stable reagent was obtained. Seaman and his co-workers (*15*) made a more thorough study of this method, and found that the stable iodine in methanol solution could be standardized against sodium thiosulfate. No evidence of significant degradative side reactions was observed, provided no great excess of iodine was added over that required to react with all the water in the flask.

Several investigators have mentioned some difficulty in finding a stopcock grease suitable for burets containing Fischer reagent. Cello-grease, Celvacene, and Cenco lubricant have been used successfully. In practice, usually sufficient pyridine hydroiodide, one of the Fischer reagent end products, diffuses around the stopcock plug to act as an excellent lubricant. A well-ground stopcock is essential. Automatic burets with a vertical stopcock plug prevent slippage.

The titration may be made visually or electrometrically. The former requires only simple apparatus and is less time-consuming; the latter is more accurate for any given quantity of water. Most of the difficulties with the visual end point are associated with the choice of a suitable color. During direct titration the color changes from canary yellow to chromate yellow and, finally, at the end point to the marked brown color of unused iodine. Often darkly colored solutions can be titrated by using a second sample as a color standard (*7, 21*). At the proper end point, the addition of 0.1 to 0.2 ml. of Fischer reagent results in a considerable deepening of the color. Most chemists unfamiliar with the end point tend to undertitrate. Unskilled operators can be trained easily for routine visual titrations. Most of the electrometric titrations are made using the dead-stop end point in which excess Fischer reagent is added and the solution is back-titrated with standard water in methanol.

Karl Fischer reagent is composed of toxic materials, but when accepted laboratory practice is followed no harmful effects should result. The reagent may be used in any well ventilated laboratory (a hood is not necessary). Spent reagent should be disposed of in a hooded area; otherwise, significant concentrations of sulfur dioxide might be passed into the atmosphere. The sensitivity of the reagent is equivalent to about 0.3 mg. of water. However, the precision and accuracy of the titration are dependent on the quantity of water in the samples and the amounts of sample employed. Those containing 20 to 40 p.p.m. of moisture require large quantities—e.g., at least 100 grams. The technique of Gester (*1*) might prove applicable in many cases. By employing ethylene glycol as an extractant, this investigator determined a few parts per million of water in hydrocarbons—for example, 250 ml. of glycol were used to extract moisture from a gallon of hexane.

APPLICATIONS

Lafferty (*5*) found that water in uranyl acetate could be determined satisfactorily. However, some uranyl phosphates, which were only slightly soluble in alcohol, did not appear to react. Direct extraction might be feasible for insoluble compounds—for example, addition of excess Fischer reagent, agitation, and back-titration with standard water-in-methanol often has proved satisfactory. In other cases inert solvents often can be found, such as acetic acid, boron fluoride complexes, and sulfuric acid (later treated with pyridine and dioxane, *12*).

Some classes of inorganic compounds, such as metal oxides, hydroxides, carbonates (except calcium), sulfites, and bisulfites, interfere with the titration for water. Usually, the interfering reaction is stoichiometric, and, therefore, the moles of apparent water found are equal to the equivalents of interfering compound. Suter (*18*) suggested that samples containing small amounts of alkali and relatively large amounts of water are conveniently titrated directly and the quantitative correction for interference applied. However, for the reverse situation Suter recommended preliminary distillation with xylene and titration of the distillate (*17*). In many cases a homogeneous azeotrope might be better suited for removal of water, provided a sufficiently high temperature could be attained to remove all the water from the sample.

Usually free water plus water of hydration is determined by Fischer reagent titration. Where only free water is desired a preliminary separation must be made. Often an extraction technique is feasible. In some cases azeotropic distillation or oven drying separates only free water. Thus both forms of water in monosodium glutamate monohydrate might be determined by Fischer reagent titration of a sample before and after vacuum oven drying.

Investigations should be continued on the application of the Fischer reagent to the determination of moisture in foodstuffs. Many important uses already have been reported. However, because of the lack of an absolute standard method, the value of the reagent in the titration of some foods has not been definitely ascertained. Morell (*10, 14*) applied the Fischer method successfully to the determination of moisture in malt, malt sirups, and miscellaneous food materials. He employed a Waring Blendor to facilitate extraction of moisture from the insoluble food into the methanol in which the sample was dispersed. Other investigators (*4, 19*) extracted moisture quickly by using methanol or excess Fischer reagent and gently refluxing for short periods of time.

Ascorbic acid is the only compound present in foods which has been shown to interfere in the Fischer titration. This material is oxidized to dehydroascorbic acid by the iodine of the reagent. However, Johnson (*4*) has reported that the quantity of ascorbic acid present in foods would indicate no more than 0.03% apparent moisture.

The Fischer reagent titration should find many uses for the determination of water in whole milk and milk products. One of the publications covering this field (*2*) indicated relatively poor precision for this titrimetric method, particularly for whole milk. Other data (*11*) demonstrated that with sufficient precautions the usual high precision of ±0.2% can be expected.

Before investigating the potential application of the Fischer reagent to titrations of commercial materials, the chemist should learn the natures of the compounds involved. This will permit him to predict likely interferences. In the organic field active aldehydes and ketones (exceptions include formaldehyde, chloral, and sugars) interfere because of slow condensation with the methanol of Fischer reagent. Diacyl peroxides are reduced by the hydrogen iodide always present in Fischer reagent. However, dialkyl and hydroperoxides do not interfere. Mercaptans (thiols) are oxidized by iodine. Methods for eliminating most of these class interferences have been described in the literature (*9*).

Several procedures for the determination of compounds other than water have been proposed which employ the Fischer reagent. Those inorganic materials which react quantitatively can be determined by direct titration if the sample is anhydrous or if an independent method is available for ascertaining the original water content. Many organic functional groups can be determined by use of quantitative reactions in which water is eliminated or absorbed. Because these procedures involve titrations for water, direct analyses often can be made of samples containing materials which interfere when analyzed by acidimetric methods. Thus, the esterification procedure for alcoholic hydroxyl is unaffected by the presence of organic or inorganic acids, easily hydrolyzed esters, or high concentrations of water. Furthermore, tertiary hydroxyl is determined quantitatively. Other analytical methods are available for carboxylic acids, anhydrides, carbonyl compounds, amines, nitriles, dialkyl peroxides, mercaptans, etc. (*13*).

Because of its nearly specific nature, Karl Fischer reagent should be considered for all analytical problems involving the determination of water. Often simple, rapid routine methods can be developed which may be used readily by nonskilled laboratory personnel.

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Determination of Carbon in Ferrous Alloys

Moderator: ROBERT M. FOWLER, *Carbide & Carbon Research Laboratories, Niagara Falls, N. Y.*
Panel: W. G. GULDNER, *Bell Telephone Laboratories, Murray Hill, N. J.*
T. C. BRYSON, *Westinghouse Electric Corporation, Pittsburgh, Pa.*
JOHN L. HAGUE, *National Bureau of Standards, Washington, D. C.*
H. J. SCHMITT, *Laboratory Equipment Corporation, St. Joseph, Mich.*

IN THE round-table discussion of the determination of carbon in ferrous alloys each speaker discussed the apparatus used in his laboratory for determining carbon in low-carbon ferrous materials, which were defined for the purpose of the discussion as metals with carbon contents <0.10%.

LOW PRESSURE COMBUSTION METHOD

Guldner described the most accurate but slowest method—the low-pressure combustion method. In general, this method consists of outgassing the crucible and then burning the sample at an oxygen pressure of about 160 mm., freezing the carbon dioxide in a cold trap, pumping off the excess oxygen, vaporizing the carbon dioxide in a known volume, and measuring the pressure with a McLeod gage. This technique, combined with magnetic transfer of the samples within the vacuum system and careful purification of the oxygen, provides a very precise analytical tool. The apparatus is a modification of that described by Wooten and Guldner (*10*). The oxygen is liquefied, passed over a catalyst, reliquefied in a double trap, and stored there until used. All stopcocks, not replaced by mercury traps, are protected by diffusion pumps or freezing traps. The combustion chamber is sealed to this trap. It is composed of a beryllia crucible surrounded by a platinum crucible, which is used to heat the sample by induction. In this method, it is possible to outgas the crucible in oxygen until the blank obtained is of an acceptable value. Then a sample which has been stored in a glass "tree" is dropped into the crucible by means of a magnet. The samples are burned in oxygen, the carbon dioxide found by combustion is frozen out by liquid nitrogen and the excess oxygen is pumped off. The carbon dioxide is then expanded to a known volume and the pressure is measured on a McLeod gage.

The method is time-consuming, but has been speeded up by Ashley and his associates at the General Electric Company, who introduced stopcocks to seal on an additional combustion chamber (*6*).

The low-pressure method is capable of giving a precision of 0.0002% carbon or better when based on a 0.5-gram sample, and thus is the best method for determining carbon in a small quantity of material. It is the most precise method for determining carbon in low-carbon alloys and most adaptable in a range of 0.02% or less. However, the method is time-consuming, and therefore not adapted to production control, the cost per determination is higher than in other methods, and the method is not generally intended for high-carbon alloys.

In a recent modification of the apparatus (*4*), an optical flat was placed in the glass over the crucible, so that temperatures in the crucible could be measured. A large wax-sealed stopcock was added to the bottom of the apparatus. In addition, a double crucible technique was developed. The platinum crucible is now surrounded by a large beryllia crucible, so that temperatures 300° or 400° C. higher can be obtained before the platinum crucible is melted. This makes it possible to burn stainless steels and many other alloys that are difficult to burn in the inner beryllia crucible without the use of a flux.

REFINED STANDARD METHOD

Bryson spoke about the normal carbon combustion furnace as set up in most field laboratories and the refinements necessary to make low-carbon determinations by this method.

Nickel boats must be preburned at a temperature well over 800° C. to control the blank. Boats are of low-carbon-nickel, 6 inches long and 0.75 inch wide. A boat will give about 50 determinations. A four-tube furnace surrounded by a metal cover connected to the outside is used. The furnace is located in an air-conditioned room and no organic solvents are permitted in this room. The Alundum combustion tubes are equipped with an automatic bell which prevents air from entering the tube when it is opened.

The combustion gases pass through sulfuric acid and a dryer tube, and are absorbed in a U-shaped Ascarite tube weighing about 60 grams. This tube is weighed on a semimicrobalance set so that one scale division is 0.01 mg. The U-tubes are platinized and the room is kept at high humidity to lower the chance of electrostatic changes on the absorption tubes. The tubes are handled with a glove and never wiped. The tube is weighed by the single deflection method at a definite time interval. All these factors are observed consistently, so that each sample is weighed in the same manner.

At Westinghouse, the standard deviation of this method is ±0.0005% carbon. Duplicates are usually within 0.00035% carbon. During the war, trouble was encountered when a number of people were working in the carbon room. Blanks were very erratic, probably because of carbon dioxide from the people in the room. This difficulty was eliminated by having only one person work in this 10 × 10 foot room.

LARGE-SAMPLE METHOD

Hague in describing the large-sample method developed at the National Bureau of Standards stated that there was not much in the way of different apparatus or gadgets. Oxygen is water-washed, preburned, run through purification bulbs, and stored in a small steel tank which acts as a reservoir for the two-tube Globar combustion furnace. Alloys requiring temperatures up to 1360° to 1375° C. are burned in preburned refractory boats. Each boat goes through a definite preburn cycle. To increase the weight of sample burned yet not increase the volume of gas to be passed, the technique of burning a number of samples and absorbing the carbon dioxide in a single bulb has been developed. The sample weight can be increased to 5 to 6 grams by this procedure.

In the dry-type train, a platinum catalyst is used to oxidize sulfur at 425° to 450° C. followed by manganese dioxide or ironized asbestos to absorb sulfur trioxide, and silica gel. Absorption bulbs are small U tubes which are light in weight yet still afford a large cross section to absorb carbon dioxide. By using several pairs of tubes in series, the completeness of absorption can be checked.

The controlling factor in low-carbon work is the precision of the blank, which must be below 0.001%. If oxygen is passed through the system for 2 days, the blank will drop to where it is satisfactory for low-carbon work. Samples are burned on a 5- to 8-minute schedule; five to six charges require 30 to 40 minutes.

Information regarding the precision was secured on the two low-carbon steel standards—a silicon steel with 0.003% carbon and a stainless steel containing 0.028%. On the lower carbon steel, the maximum deviation from the average was on the order of 0.0005% which is about the difference due to accidental variations in the blank. The blank apparently fairly well controls the precision one can get on samples. These were especially prepared samples; a great number of samples will not agree so well.

CONDUCTIVITY

In describing the conductivity method (*1*), Fowler stated that his group had developed a method originated about 30 years ago based on the change in conductivity of a dilute barium hydroxide solution. The biggest problem was designing an absorber. The absorber as it has evolved consists of a thermostated water-jacketed circulating glass frit absorber containing the 15-mm. square platinized electrodes and an identical set of reference electrodes also in barium hydroxide solution. These are connected to an amplifying Wheatstone bridge circuit which uses a distorted cathode ray tube as an alternating current galvanometer. The bridge was designed and constructed by J. H. Harley, who is now with the Atomic Energy Commission. To use barium hydroxide solution, it was necessary to add modification agents. The present solution contains 1 gram of barium hydroxide, 5 ml. of octyl alcohol, and 1 gram of gelatin per liter.

With this absorber, a Globar furnace and a dry-type train are used. Inasmuch as only changes in conductivity are measured, the blank can be large only if constant, because the apparatus is standardized by burning portions of standard steels and plotting change in conductivity *vs.* carbon content. Actually the blank can be kept down to the order of 0.001% carbon on 0.5-gram samples.

Size of sample has been reduced because with high-chromium materials better burns are secured. As temperatures increase, refractory life is shortened, so that another method of heating such as high frequency would be desirable.

The standardization curve approaches a straight line up to about 0.1% carbon, but falls off badly at 0.2%. By varying the concentration of barium hydroxide, the range of operation can be shifted. The curve shifts slightly from day to day, so is redetermined at least twice a day.

With this apparatus, it is possible to watch the carbon dioxide being evolved. The fact that with some materials evolution of carbon dioxide is not complete in 5 minutes with oxygen rates of 6 to 800 ml. per minute means that extravagant claims for 5-minute carbons are to be viewed with suspicion; certain materials will not give up the carbon dioxide so rapidly.

Aluminum oxide bedding materials contain sufficient alkali that, if they are heated to the temperature of burning steel for a few seconds and then ground in air, they will give up carbon dioxide at 1425° C. As this cycle can be repeated indefinitely, slugs left after combustion cannot be reground if aluminum oxide was used as a bedding material.

Combustion tubes also absorb and desorb carbon dioxide, and so must be protected from the air by Ascarite. Oxygen is preburned and purified and only preburned boats are used.

As for precision, results on the two standards mentioned above were satisfactory but a shade low. Can this be due to something else in steels which is absorbed by Ascarite? Tests for sulfates and nitrates on exhausted Ascarite have been inconclusive; the difference is of the order of 0.0005 to 0.001% carbon. On very low-carbon steels, a very dilute barium hydroxide solution could be used and results of high precision secured. The time required is about 10 minutes per determination after the absorber is standardized.

VOLUMETRIC CARBON APPARATUS

Schmitt described the volumetric carbon apparatus. The essential parts are the combustion furnace operating at 2400° F. with a 0.75-inch diameter tube because of the limited capacity of the analyzer. The sample, in a zirconium silicate boat without bedding and with tin as an accelerator, is placed in the tube. Alloy steels are preheated, but carbon steels are not. The oxygen goes through the normal purifying train, and, in some special cases, has been passed over hot copper oxide to remove traces of hydrocarbons.

The exit gases consist of carbon dioxide, excess oxygen, sulfur dioxide, and water. Sulfur dioxide is removed with manganese dioxide and the other gases go to a 550-ml. receiving bulb. The volume is measured and then the gas is passed through potassium hydroxide solution and when the carbon dioxide is absorbed, the change in volume is read as per cent carbon. This apparent percentage is corrected for temperature and barometric pressure. Final leveling of the Orsat is by means of an air pressure system. The system is water-jacketed with a circulating pump to equalize the temperature. To reduce temperature effects further, a compensating buret is employed and the measured volume is corrected for any changes in the compensating buret.

Two steel mill laboratories that are using this method, three shifts per day, claim 5-minute carbons including weighing and a precision of ±0.002% carbon on high-silicon and stainless steels. In a precision test over 7 days on over 100 samples of standards, the average deviation was 0.0011% carbon. With an experienced operator the results improve, as perhaps the greatest single variation in the operation of the instrument is the ability of the operator to get used to it. With special samples, harder to burn, more than 5 minutes are required at a higher temperature and slower oxygen flows. To get results within ±0.0006% carbon, about 10 to 14 minutes are required per analysis.

DISCUSSION

In the general discussion period that followed it was pointed out that a disadvantage of the volumetric and conductivity methods is the necessity of controlling the temperature. On the other hand, a glass blower is required to maintain the low-pressure apparatus. A Linde Air Products Company V-11 regulator was mentioned as a desirable low-pressure regulator for controlling the oxygen flow. A constant flow of oxygen is desirable in the conductivity method.

A titrimetric procedure, an adaptation of that of Smith and Niederl (*8*), is used with a measured amount of barium hydroxide solution, and the excess is titrated with 0.05 *N* hydrochloric acid. The procedure is standardized empirically using a microburet. The reproducibility is excellent.

Use of a sodium hydroxide solution with added indicator for absorbing carbon dioxide was described (*9*). The decrease in the intensity of the color of the sodium hydroxide solution was correlated with carbon content. Although an adequate absorber could not be secured, a degree of success was secured on low-carbon–silicon steels at the 0.01% carbon level. Several indicators were tried, and it was observed that with some materials there was an immediate decrease in color, whereas when other materials were burned, the carbon dioxide came over in dribbles.

The titrimetric procedure was used many years ago in the steel industry and for organic compounds. The speaker liked it because it was unaffected by humidity and electrostatic changes and largely independent of temperature.

The question of the absolute accuracy of carbon determinations was raised by a speaker who asked how one can tell if all the carbon is oxidized and evolved. In reply, a second speaker described the National Physical Laboratory method of dissolving a steel in copper-potassium chloride and burning the residue (*5*). In comparison with the low-pressure method, the results agreed fairly well in the case of four samples, but on a fifth were not satisfactory. This method is independent of burning. The chairman stated that this method was widely used in the steel industry 75 years ago, but he doubted if it would give satisfactory results on alloy steels.

A speaker mentioned the case of a chromium carbide which in the standard combustion procedure showed only 0.01% carbon, yet on the low-pressure apparatus with higher temperatures showed 8 to 9%. The carbide simply could not be burned at the lower temperature at which the standard combustion procedure was run.

In one case, experiments with burning carbides at 1100° C. and up to 1425° C. each time gave a higher carbon value. The experiments were stopped at 1425° C. because the combustion tubes sagged. Presumably, if one could operate at 1500° C. a higher value might be obtained. Guldner stated that with his apparatus it was always possible to get a rapid burn and temperatures were probably well above 1600° C.

It was suggested that radioactive carbon be introduced into a steel and the activity after combustion be correlated with the carbon content. Hague described plans for making this experiment in the near future.

One speaker stated that, in his experience, time is also a factor nearly as important as temperature. At a lower temperature for a longer time, one can get all the carbon out.

It was pointed out that it would be very interesting to see if the combustion tube acquired any activity due to adsorption of active carbon dioxide by the surface of the tube.

The gas in absorption tubes at the time of weighing makes no difference, so long as the procedure is exactly the same each time.

As for the variation between large and small chips, even low-carbon materials are not homogeneous and with cast irons the problem is especially difficult. One solution suggested was to take a 0.25-inch rod sample and burn with tin, or use the procedure of Deas (*3*).

Magnesium-treated cast irons often give off an odor similar to commercial acetylene, and one speaker wondered if appreciable carbon was lost by this reaction with moisture in the air. An article in *Foundry* (*2*) states that, in analyzing such irons, the results were 0.1 to 0.2% lower on chips than on solid pieces.

Experiences in the sampling of cast iron were described by another speaker. On 2 × 2 × 1 inch blocks, variations between two laboratories were as much as 0.3% carbon; so a study was made of the variation of carbon with depth of drilling. The carbon content of the center of the block was as much as 0.3% higher than the outside. As a result, the present procedure is to cast in a chill mold, and drill the sample from the top to the center of the casting. This procedure gives the most uniform sample. However, if the sample is small enough to be entirely burned, it may not be representative. In trying to get away from loss of graphite through dusting, chemists have fallen into the larger error of not getting a representative sample of the whole iron. This speaker reported very good checks between the drilled and mixed samples and solid samples obtained by breaking off pieces.

Another speaker stated that the simplest way to sample cast iron is to cast a self-chilled plate and break it up in a mortar. In this way, checks to 0.01 to 0.02% can be secured.

The question of segregation in the liquid iron was raised, and the answer was that slices were taken from the casting to check with the chilled sample. If the section is uniform, the frozen cross section should represent the molten sample. The only way to handle the problem is to come as close to a cross section of the sample as possible, because there is always segregation.

Schmitt asked whether chemists would prefer to measure to 0.1 ml. or weigh to 0.2 mg., inasmuch as each measurement represents the same amount of carbon dioxide. He contended that it is far easier to measure to 0.1 ml. than to weigh to 0.2 mg. The chairman answered that theoretically the weighing is far more accurate but, practically, it depends on what the chemist is trying to do.

A speaker mentioned the U. S. Steel Corporation method where the carbon dioxide is absorbed in sodium hydroxide and the excess is back-titrated (*7*). This method has given good results in one laboratory.

The use of desiccants with Ascarite was discussed, and the consensus was that Ascarite must be followed with a desiccant. Some improvement in the quality of Ascarite was noted. Experience indicated that Caroxite was very similar to Ascarite. One speaker reported good results with the carbon dioxide absorbent produced by the G. Frederick Smith Chemical Company, especially for micro work. The chairman commented that trains without liquids appeared to be more satisfactory than those containing aqueous scrubbers. It appears to be a questionable procedure to wet a gas, dry it, then wet it again, and try to not get any water in the absorber; it is better to dry it and keep it dry.

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NOTES ON ANALYTICAL PROCEDURES . . . .

Measurement of P^{32} in Plant Material by the Use of Briquets

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A ROUTINE quantitative procedure for the determination of P^{32} in plant material has been described by the writers (*2*). This procedure entailed the separation of the phosphorus from the plant material, its precipitation as magnesium ammonium phosphate hexahydrate, and its subsequent collection as a uniform layer on a specially prepared filter.

A faster routine quantitative procedure became desirable when it was necessary to analyze ever-increasing numbers of vegetative samples from greenhouse and field experiments designed to study the utilization of phosphorus from fertilizers labeled with P^{32}. A procedure, therefore, has been established for the quantitative determination of the P^{32} activity of plant material by the use of briquets, thereby eliminating time-consuming chemical precipitations and separations. The same degree of precision as in the precipitation technique is maintained.

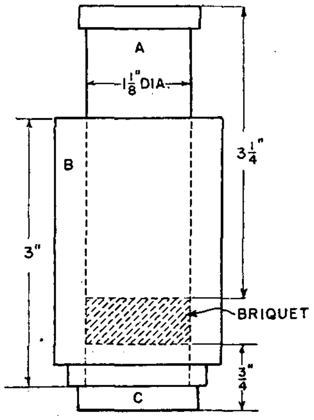

Figure 1. Form for Making Briquets Used to Measure P^{32} Activity in Plant Material

PREPARATION OF PLANT MATERIAL BRIQUETS FOR RADIOACTIVE MEASUREMENT

The samples of plant material containing P^{32} are dried at 65° C. and ground to pass through a 20-mesh screen.

The form used for making the briquets (Figure 1) is of cylinder-plunger type and is composed of plunger *A*, cylindrical form *B*, and bottom or base for the briquets, *C* (manufactured by Fred S. Carver, Inc., 345 Hudson St., New York 14, N. Y.). All parts are machined to fit very closely.

To prepare a briquet, parts *B* and *C* of the form are assembled. Approximately 5 grams of the dried, ground plant material are then weighed and transferred to the form, after which part *A* is fitted into place. A pressure of 12,000 pounds per square inch is applied to the plant material for about 3 minutes by means of a laboratory hydraulic press. After the required time the pressure is released and the briquet is removed from the form. When *C* is removed the briquet is readily pushed out through the bottom of the form with the plunger, *A*. Briquets made under these conditions are cylindrical in shape, having a diameter of 1.125 inches and a height of approximately 0.25 inch (0.6 cm.). They are sturdy and easily reproduced and their dimensions are suitable for radioactive measurements.

The briquetted plant material sample can be conveniently numbered with India ink. Contamination of laboratory equipment and samples was controlled by frequent cleaning with a vacuum cleaner.

MEASUREMENT OF RADIOACTIVITY

The activity measurements of the briquetted plant material samples containing P^{32} are made by the use of a special holder, which is designed to fit counter mounts used in this laboratory. Figure 2 is a cross-sectional view, showing the holder with briquet in position for counting. Constant geometry is maintained by pressing the top surface of the briquet against a guard plate, thus always keeping the top of the briquet at a constant distance from the counter tube window. An opening 1 inch in diameter is provided in the guard plate. However, if it is necessary to reduce the counting rate, an aluminum filter may be substituted.

Sample changing is accomplished by lowering the briquet away from the guard plate and locking the holder in this position. The briquet holder is then removed from the counter mount and a new briquet is inserted.

INFINITE THICKNESS OF COMPRESSED PLANT MATERIAL FOR P^{32}

The infinite thickness of a radioactive material is that thickness of the material beyond which there is no increase in measured activity. To determine the actual amount of the plant material containing P^{32} which contributes to the measured activity of a briquet, increasing amounts of a radioactive plant sample were made into briquets and the activity was measured in the manner described above. The results of the experiment are shown in Figure 3. Constant activity was obtained with samples of 3 grams (thickness approximately 470 mg. per sq. cm.) or more. By using at least 5 grams (thickness approximately 784 mg. per sq. cm.) of plant material as a sample all radioactive measurements can be safely made at infinite thickness and sample thickness corrections are thereby avoided.

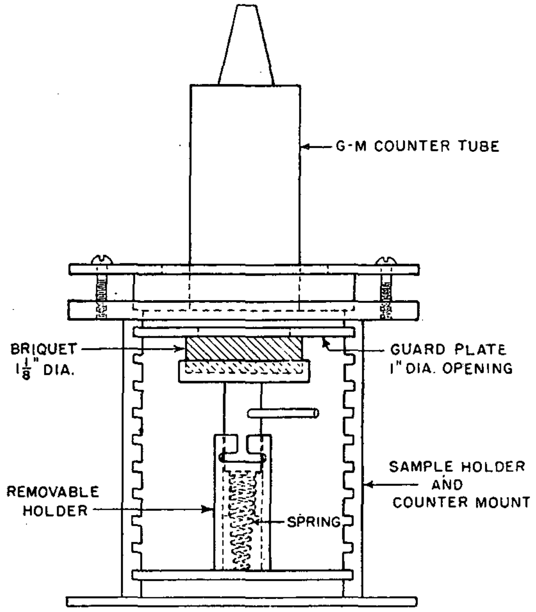

Figure 2. Support with Briquet in Position for Counting

To determine the relationship of concentration of P^{32} in compressed plant material to the measured activity, plant material containing P^{32} was diluted by intimate mixing with different amounts of "nonradioactive" or check plant material. Five grams of each dilution were compressed into a briquet and the activities were measured. These activities when plotted against the per cent radioactive material in the sample (see Figure 4) gave a linear function.

ACTIVITIES OF BRIQUETS AND OF PRECIPITATES

A comparison of the specific activity, $P^{32}/(P^{31} + P^{32})$, of the fertilizer preparation with that of the plants grown on a soil receiving this fertilizer enables estimation of the proportion of the phosphorus contained in the plants derived from this fertilizer. Having established a reproducible method for the direct measurement of P^{32} in plant material, it remained necessary to compute a specific activity of the plant material which can be compared with that of the original fertilizer material. Because the specific activity of the fertilizer material is most conveniently measured by separating the phosphorus from the material and precipitating it as magnesium ammonium phosphate, the specific activity of the plant material should be measured under similar counting conditions.

The ratio of the activities of plant samples measured as briquets to the activities of magnesium ammonium phosphate hexahydrate precipitates separated from these same samples, is a constant. Table I gives the radioactivity measurements of plant material samples both as briquets and as precipitates and the resulting relationship. The briquets were prepared as described above and the precipitates of the plant material were prepared from 2.00 grams of plant material as described previously (*2*). The factor for the conversion of briquet activities to that of the magnesium ammonium phosphate precipitate from 2.00 grams of plant material was 1.805. The amount, 2.00 grams of plant material, was chosen arbitrarily. Any convenient amount of plant material could have been chosen for the determination of a subsequent conversion factor.

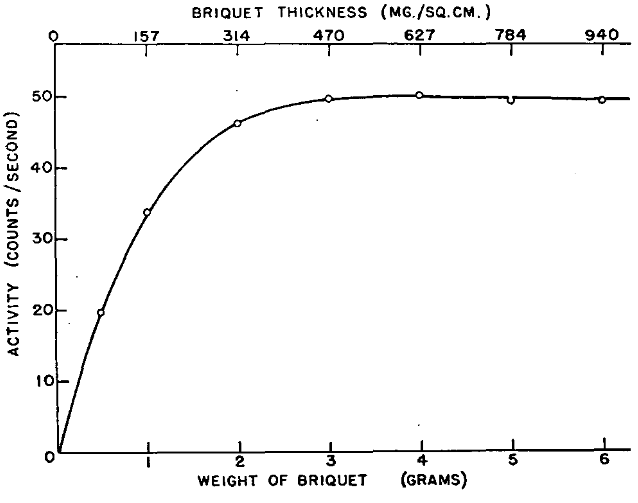

Figure 3. Infinite Thickness of Briquets Prepared by Compressing Ground Plant Material Containing P^{32}

The specific activity of the plant material may now be calculated from the measured activity of the briquet and at the same time be comparable with the specific activity of the original fertilizer material measured as a magnesium ammonium phosphate hexahydrate precipitate. The specific activity of a plant material sample may be expressed as:

$$\text{Specific activity} = \frac{\text{measured briquet activity} \times 1.805}{\text{total phosphorus in 2.00 grams of sample}}$$

Total phosphorus in the plant material was determined by Mission's vanadomolybdate method (*1*, *3*).

Table I. Determination of Conversion Factor

(For conversion of briquet activity to activity given by $MgNH_4PO_4.6H_2O$ precipitate from 2.00 grams of same plant material)

Sample No.	Activities[a] Magnesium ammonium phosphate ppt. from 2. 0 grams of plant material	Sample as briquet	Factor
	Counts/sec.	*Counts/sec.*	
4MA	28.54	15.81	1.805
4M	29.64	16.14	1.836
5LA	10.69	5.92	1.806
57A	25.70	14.25	1.804
60A	20.59	11.54	1.784
76A	35.71	19.92	1.793
		Av.	1.805

[a] Corrected for background and radioactive decay. Recovery time corrections were insignificant.

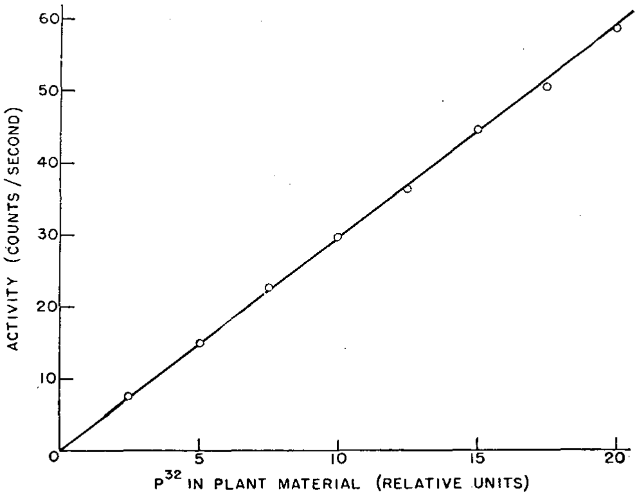

Figure 4. Relation of Concentration of P^{32} in Plant Material to Observed Activity of Briquets

It is shown in Figure 3 that the amount of plant material which contributes to the activity of a briquet is approximately 3.00 grams. The results of Table I indicate that the precipitation procedure is about 2.7 times as sensitive as the briquet procedure. The greater sensitivity of the precipitation procedure, however, is compensated for by the greater speed of the briquet procedure.

INFLUENCE OF K^{40} ON BRIQUET ACTIVITIES

The naturally radioactive isotope of potassium, K^{40}, can be expected to contribute some activity to the briquets, but it does not create any difficulty except when the P^{32} activity is low. If this occurs the radioactivity measurements should be made by a precipitation technique (*2*). Activities due to K^{40} in briquets of plant material have been measured and found to be about 0.1 count per second. Thus, with plant material of relatively high activities, the activity due to K^{40} becomes insignificant. Briquets of plant material from check treatments are, however, usually used in making background determinations. In this manner the correction for natural background and the activity due to the K^{40} are determined in one operation. The natural background activity using 2 inches of lead as a shield for the counter assembly employed in this laboratory is 0.25 count per second.

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Estimating Oil Yield of Oil Shale from Its Specific Gravity

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THE specific gravity of oil shale may be used as a rapid and practical method of estimating the oil yields of shales from a given source. A study of 32 oil-shale samples of the Green River formation from the Bureau of Mines oil-shale mine (*3*) at Rifle, Colo., showed that a relationship existed between the oil yields of these shales determined by the modified Fischer-assay method (*4*) and their specific gravities. This oil yield–specific gravity relationship provides a convenient means of estimating the oil yields of similar shales from the same source. A further examination of 38 other domestic and foreign oil shales indicated that similar but numerically different relationships may be used to estimate the oil yields of shales from other specific sources.

The modified Fischer assay is the present accepted method of determining the oil yield of oil shale (*4*). By this method, the oil shale is retorted in a special cast-aluminum retort, and the oil yield is determined by condensing and measuring the volatilized oil. An assay requires approximately 2 hours for completion, whereas, by the proposed method, several specific gravities can be completed in 1 hour in a manner similar to that prescribed by the American Society for Testing Materials for determining the true specific gravity of burned refractory materials (*1*). From these specific gravities, the oil yields are estimated from a graph of the oil yield–specific gravity relationship established from representative samples of the particular deposit. The estimated oil yields are not as reliable as values obtained directly by the assay of the samples. However, they are accurate enough for certain control purposes in mining and processing oil shale, for which rapid results are desired.

This method of approximating the oil yield of oil shale is similar to that proposed by Engelhardt for estimating the organic content of Estonian kukkersite (*2*). Engelhardt showed that the relationship between the specific volume (volume of 10.0 grams) of kukkersite and its organic content was nearly linear and gave an equation whereby the organic content could be calculated from the specific volume. The oil yield–specific gravity relationship for the Green River shales investigated was not linear, primarily because of variations in the inorganic materials in the different grades of shale.

PROCEDURE

Approximately a 10-gram sample of the dry shale crushed to pass an 8-mesh-per-inch sieve is weighed accurately into a 25-ml. Hubbard-Carmick pycnometer. Kerosene of known specific gravity, which has been topped to approximately 392° F. (200° C.), is added to cover the sample. The mixture is stirred with a glass rod, and the shale particles adhering to the glass rod are washed carefully into the pycnometer with a small portion of the kerosene. Air bubbles in the mixture are removed by suction (that obtained from a water aspirator is sufficient), after which the pycnometer is filled with kerosene and placed in a water bath maintained at 60° ± 0.1° F. Additional kerosene may be required after cooling for several minutes. When the pycnometer and contents have attained constant temperature (about 20 minutes), the stopper is inserted firmly, and the excess kerosene is removed with a dry cloth. The pycnometer is transferred to an ice bath to shrink the volume of kerosene in the pycnometer, then washed with a stream of acetone and dried. Finally, the pycnometer is weighed and the specific gravity of the oil shale is calculated. The oil yield of the shale is then approximated from the specific gravity by an oil yield–specific gravity curve (similar to that given in Figure 1 for oil shales from the Bureau of Mines oil-shale mine) established for the particular oil-shale deposit.

EXPERIMENTAL

The 32 samples of oil shale from the Green River formation at the Bureau of Mines oil-shale mine used in this study were chosen to represent the different grades of oil shale likely to be encountered in that area. The modified Fischer oil yields of the samples ranged from 10.0 to 76.6 gallons of oil per ton of shale and their specific gravities at 60°/60° F. ranged from 2.544 to 1.668.

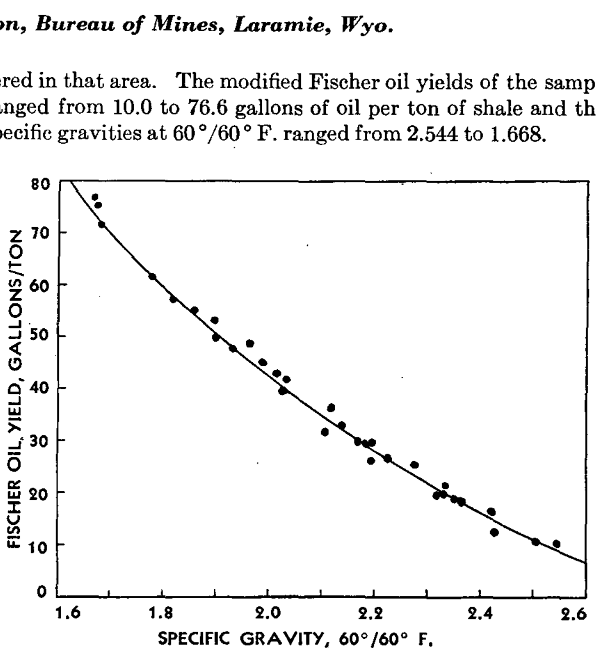

Figure 1. Relation of Specific Gravity to Fischer Oil Yield of Colorado Oil Shale

Figure 1 shows the curve obtained by plotting the oil yields of the samples against their specific gravities. From this curve, the oil yield of each sample was estimated. The estimated oil yields differed from the actual oil yields by an average of 1.2 gallons of oil per ton of shale. The maximum difference between the determined and estimated oil yields was 3.0 gallons of oil per ton or an average of 3.9%. The values for the individual samples are given in Table I.

Table I. Specific Gravity and Oil Yield of Oil Shale from Vicinity of Rifle, Colo.

Sample No.	Specific Gravity, 60°/60° F.	Modified Fischer Oil Yield, Gal./Ton	Estimated Oil Yield, Gal./Ton	Difference[a] Gal./Ton	Difference[a] %
1	2.544	10.0	9.5	0.5	5.0
2	2.504	10.5	11.0	0.5	4.8
3	2.428	12.4	14.7	2.3	8.5
4	2.419	16.4	15.2	1.2	7.3
5	2.364	18.2	18.2	0.0	0.0
6	2.352	18.9	18.8	0.1	0.5
7	2.333	21.1	19.8	1.3	6.2
8	2.327	20.0	20.0	0.0	0.0
9	2.322	19.6	20.5	0.9	4.6
10	2.272	25.3	23.5	1.8	7.1
11	2.224	26.5	26.5	0.0	0.0
12	2.193	26.1	28.7	2.6	10.0
13	2.193	29.8	28.7	1.1	3.7
14	2.183	29.6	29.5	0.1	0.4
15	2.168	29.8	30.5	0.7	2.3
16	2.139	32.9	32.5	0.4	1.2
17	2.116	36.3	34.0	2.3	6.3
18	2.107	31.7	34.7	3.0	9.5
19	2.030	41.8	40.5	1.3	3.1
20	2.027	39.6	40.7	1.1	2.8
21	2.015	42.9	41.5	1.4	3.3
22	1.986	45.1	43.7	1.4	3.1
23	1.962	48.6	45.7	2.9	6.0
24	1.929	47.7	48.7	1.0	2.1
25	1.899	49.9	51.2	1.3	2.6
26	1.895	53.4	51.5	1.9	3.6
27	1.856	55.2	55.0	0.2	0.4
28	1.817	57.1	58.7	1.6	2.8
29	1.778	61.8	62.7	0.9	1.5
30	1.680	71.7	72.5	0.8	1.1
31	1.673	75.5	73.2	2.3	3.0
32	1.668	76.6	74.0	2.6	3.4
			Average difference	1.2	3.9
			Maximum difference	3.0	10.0

[a] Difference between oil yield by modified Fischer-retort method and estimated from specific gravity of oil shale.

Table II. Specific Gravity of Foreign and Domestic Oil Shales

Sample No.	Source	Modified Fischer Oil Yield, Gal./Ton	Specific Gravity, 60°/60° F.
51	South Africa	33.3	1.645
52		45.0	1.599
53		45.6	1.627
54		68.8	1.515
55		99.8	1.376
56	Australia	26.2	2.072
57		82.9	1.633
58		119.7	1.301
59		135.9	1.234
60		147.2	1.256
61	Brazil	31.3	1.962
62		33.6	2.016
63	Manchuria	26.1	1.908
64		26.3	1.915
65		38.6	1.539
66		48.1	1.687
67		55.9	1.570
68		56.8	1.553
69	Indiana	4.8	2.536
70		8.6	2.183
71		9.7	2.216
72		12.4	2.146
73		14.3	2.212
74	Kansas	10.9	2.133
75		8.1	2.207
76	Kentucky	5.6	2.367
77		11.5	2.232
78		16.4	2.114
79	Nevada	10.2	2.356
80		22.8	2.072
81	Ohio	7.1	2.490
82		9.8	2.382
83		14.7	2.222
84	Tennessee	52.8	1.520
85	Texas	3.7	2.359
86		7.3	2.332
87	Wyoming	7.4	2.354
88		39.8	1.969

Table II gives the oil yields and specific gravities of 38 oil shales from four foreign countries and eight states other than Colorado. These data show that the numerical value of the oil yield–specific gravity relationship for the specified Colorado oil shales is not applicable to all oil shales; however, they indicate that similar relationships may be obtained for oil shales from other specific areas. Furthermore, this rapid method of estimating the oil yield of shale may be useful for routine control purposes where time and equipment will not allow the determination of oil yields by the modified Fischer assay.

SUMMARY

The relationship between the oil yield by the modified Fischer assay and the specific gravity of certain Colorado oil shales provides a practical means of estimating the oil yields of other oil shales from the same area. The oil yields estimated from the specific gravities of samples from the Green River formation in the Bureau of Mines oil-shale mine, Rifle, Colo., differed from the actual oil yields of the shales by a maximum of 3.0 gallons of oil per ton and by an average of 1.2 gallons of oil per ton of shale. The numerical value of the oil yield–specific gravity relationship for these Colorado oil shales was not applicable to all oil shales. However, it indicated that similar relationships may be obtained for oil shales from other specific areas, and this method of estimating the oil yield of oil shale may be adapted to rapid routine testing of oil shales where time and equipment will not allow assays to be made by the more reliable modified Fischer-retort assay method.

This report represents the results of work done under a cooperative agreement between the Bureau of Mines, United States Department of the Interior, and the University of Wyoming.

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Acid-Bleached Fuchsin Solution as Analytical Reagent

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Diluted solutions of basic fuchsin, which are decolorized with sulfuric acid and to which formaldehyde is added after bleaching, have been recommended as a quantitative reagent for sulfur dioxide and thiols. The reagent is much less sensitive for thiosulfate. Other substances than thiols interfere with the sulfur dioxide reaction. Their reactions are less sensitive and slower for comparable concentrations than those of the thiols, but they are also more difficult to mask. The masking of thiols or thiosulfate can be achieved with mercuric chloride or with the chlorides of platinum and palladium(II). The mercury-precipitated thiols and thiosulfates must be removed by filtration or centrifugation before the sulfur dioxide test is carried out; otherwise the sulfur dioxide reagent will react with the precipitate. The mercuric thiosulfate precipitate is even more reactive than thiosulfate itself, whereas the platinum and palladium precipitates are not reactive or much less so.

Grant (*1*) was the first to point out unknown substances which interfere with the sulfur dioxide reaction and which, unlike the thiols, cannot be removed by masking with mercuric chloride. He removed the mercuric thiol precipitates by centrifugation before adding the sulfur dioxide reagent, which can be kept for a long time by working with two stock solutions.

Stock Solution I is made from 228 ml. of water, 22 ml. of concentrated sulfuric acid, 4 ml. of basic fuchsin (3% alcoholic solution), or 8 ml. of fuchsin in the case of Grant's quantitative procedure. The solution is made up with water to 400 ml. and aged 3 days. The precipitate which forms is decanted or filtered off, or, better still, the fresh solution is shaken with 150 ml. of carbon tetrachloride, when the colloidal precipitate goes into the interface. This purification procedure with the aid of a separation funnel is useful under all similar circumstances.

Stock Solution II is made by adding 5 ml. of 40% formaldehyde, to water to make 100 ml.

Reagent. Before use, 10 parts of I are mixed with 1 part of II. A sensitive reagent for ascorbic acid is obtained by taking 6 parts of II.

Grant (*1*) makes the interesting statement that the blanks of the tissues which he tested for sulfur dioxide give a slightly positive color reaction, although "it seems unlikely that it represents any naturally occurring sulfur dioxide." The author's experiments with gelatin, for which he introduced the test, have also shown interference by gelatin, even in the case of Eastman electrodialyzed gelatin and other gelatins that contained no sulfur dioxide. The interfering gelatin reaction is, however, much less sensitive and much slower than the reaction with sulfur dioxide. It could be still further subdued and almost inhibited by platinum chloride, and although no amino acids present in gelatin were found to give the interfering reaction, cysteine and tryptophan were recorded as interfering with the sulfur dioxide test. The tryptophan trace reaction is, however, very slow. Enediols like ascorbic acid and reductone and *o*-dihydroxybenzenes like adrenalin, catechol, and ethyl hydrocaffeate, were found to give strong but slow to very slow color reactions with the reagent. These reactions, unlike the quick but fading thiol reaction of cysteine, are not inhibited by mercuric or platinum chloride, and it can therefore be assumed that untreated fruit juices or proteins which give the color reaction very distinctly, even in the presence of platinum or mercuric chloride, contain ascorbic acid, an enediol, or *o*-dihydroxybenzene derivatives. The reaction with proteins needs further investigation.

The presence of anionic wetting agents can also interfere with all the reactions which are given by acid-bleached fuchsin-formaldehyde (sulfur dioxide, thiols, enediols, *o*-dihydroxybenzenes, sulfinic acids), but the anionic wetting agents are easily detected because they react slowly with Stock Solution I, and do not depend on the presence of formaldehyde.

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Microdetermination of Iodine in Plant Material

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THE simplicity inherent in the spectrophotometric procedure used by Gross, Wood, and McHargue (*1*) for the determination of iodine in biological materials makes it well suited for the routine examination of agricultural crops. However, certain modifications which make it possible to determine smaller amounts of iodine extend greatly the usefulness of this procedure.

Table I. Determination of Iodine

Iodine, γ/5 Ml.	% Transmittance at 575 mμ
0.10	96.7
0.25	92.0
0.50	84.0
1.00	70.0
2.50	41.0
5.00	17.0

EXPERIMENTAL

By decreasing the final volume from 50 to 5 ml., 0.1 microgram of iodine can easily be detected when a Model DU Beckman quartz spectrophotometer is used with standard Corex cells having a 1-cm. light path. However, the color developed is not suitably stable if the concentrations of the reagents are maintained at the level used by Gross, Wood, and McHargue. The chief difficulty appears to be associated with a slow decomposition of the potassium iodide, resulting in a gradual increase in the amount of starch-iodide chromogen formed. This difficulty is partially overcome by greatly decreasing the amount of potassium iodide used. Further improvement is obtained by substituting phosphoric acid for sulfuric acid in order to maintain the pH of the solution at approximately 2.5. These changes do not significantly decrease the sensitivity of the starch to iodine and the peak of maximum light absorption remains at 575 mμ. The color is stable for an indefinite time if the solution is kept in a bath of ice water until transmittance readings are ready to be made. Cooling also increases the sensitivity of the starch iodide reaction, especially at the lower concentrations of iodine. Potato starch appears to be slightly better than arrowroot starch, because the sol is less opalescent.

PROCEDURE

A sample of dry plant material weighing 1 gram will be sufficient in most cases. Oxidize the sample in the usual manner, using 10 ml. of 10 *M* chromium trioxide solution and 50 ml. of concentrated sulfuric acid. After cooling the digestion mixture add 50 ml. of distilled water and 2 or 3 large chips of porous tile or alundum.

Collect the distillate in a 50-ml. Pyrex beaker containing 1 ml. of 0.2 *N* sodium hydroxide. Five milliliters of 30% phosphorus acid are sufficient to reduce the excess chromium trioxide completely and ensure recovery of the iodine present. Collect exactly 35 ml. of distillate.

After the distillate has been evaporated to 5 or 10 ml., prepare a reference solution in another 50-ml. Pyrex beaker by adding 1 ml. of 0.2 *N* sodium hydroxide and 5 to 10 ml. of water. Oxidize the iodides as described by Gross, but use 1 or 2 drops of 0.2 *M* potassium permanganate followed by 3 drops of 28% phosphoric acid, and continue the evaporation to a final volume of 2 or 3 ml.

Transfer the contents of the beakers to small graduated cylinders or test tubes marked at 5 ml. Immerse them in a beaker of ice water for 2 minutes. Add one drop of 5% potassium iodide, mix, and add 10 drops of 0.25% potato starch. Mix thoroughly, dilute to the 5-ml. mark, mix again, and immerse in the ice water. Allow the tubes to remain in the ice water at least 2 minutes before reading the transmittance of the unknown against the reference solution. Make readings at 575 mμ in a cell having a 1-cm. light path.

A curve prepared in the following manner indicates that the starch-iodide chromogen follows Beer's law over the range of 0.1 to 5.0 micrograms of iodine. Standard solutions of potassium iodate containing from 0.10 to 5.00 micrograms of iodine and 1 ml. of 0.2 *N* sodium hydroxide are treated as described above for an unknown, except that distillation and the treatments prior to distillation are omitted. When the micrograms of iodine per 5 ml. are plotted against the logarithm of the per cent transmittance a straight line is obtained which passes through 100% transmittance at zero microgram of iodine. Table I shows the results obtained in this laboratory with a Model DU Beckman spectrophotometer.

Table II. Recovery and Reproducibility Tests

Material	Known Iodine Content	Found by This Method	
	γ	γ	*P.p.m.*
Standard iodine solution	0.25	0.24	...
Standard iodine solution	0.50	0.49	...
10 ml. of 10 *M* CrO_3	..	0.18	...
30 ml. of 10 *M* CrO_3	..	0.55	...
Wheat straw I, 5-gram sample	..	1.32	0.264
Wheat straw I, 1-gram sample	..	0.25	0.250
Wheat straw II, 1-gram sample	..	2.85	2.850

If commercial chromium trioxide is used in this procedure, it is necessary to make a blank correction or to prepare a reference curve by carrying the standard iodine solutions through the same procedure used for an unknown. The latter procedure is preferable, for in this case the iodine standards and unknown receive identical treatments. The reference curve obtained in this manner will deviate from the one shown in Table I, depending on the amount of iodine in the digestion and distillation reagents. A new curve should be prepared with each new batch of chromium trioxide solution. However, this is not inconvenient, because 2 liters of this reagent are enough for 200 determinations.

If commercial chromium trioxide sufficiently low in iodine cannot be obtained, the procedure used by Matthews, Curtis, and Brode (*2*) for preparing this reagent is recommended.

The average error in 20 determinations (each performed in triplicate) was ±0.02 microgram and was independent of the amount of iodine in the range of 0.10 to 5.00 micrograms.

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Estimation of Vitamin A in the Presence of Interfering Materials

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IN THE estimation of vitamin A by measurement of the absorption at 325 mμ, a correction must usually be made for the presence of other substances which absorb in this region, and a number of methods have been introduced to make allowance for this irrelevant absorption. Morton and Stubbs (*1*) have recently investigated the nature of the correction required for various fish oils and have introduced a correction procedure based on measurements of the extinction coefficients at $\lambda_{\max.}$ and at two other wave lengths such that, for pure vitamin A, E is $^6/_7$ of $E_{\max.}$ From the difference between these two extinctions and the extent to which they deviate from $^6/_7$ of $E_{\max.}$, the irrelevant absorption at $\lambda_{\max.}$ may be calculated.

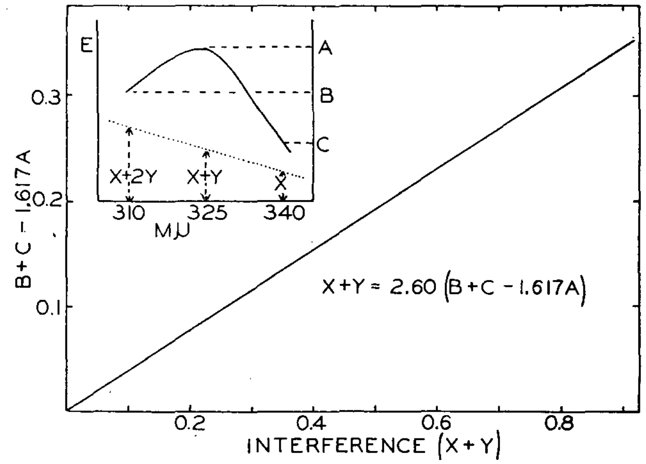

Figure 1. Application of Correction Procedure

A similar method, but one which lends itself to a simpler calculation, has been in use for some time in this laboratory for detecting and estimating vitamin A in the presence of partially oxidized carotene. This method, in which readings are taken at $\lambda_{\max.}$ and at two other wave lengths equidistant above and below it, is capable of extension to other examples of superimposed irrelevant absorption, provided the points corresponding to the three wave lengths on the curve for the interfering substances alone lie in a straight line. Over the small wave band considered, this generally applies and the proviso is common to all such three-point methods.

The wave lengths at which readings are taken and the interpretation of these readings will depend on the solvent employed and on whether the vitamin is free or esterified, inasmuch as these factors affect both the position of $\lambda_{\max.}$ and the shape of the absorption curve of the vitamin. The application of the correction procedure to vitamin A alcohol plus interfering materials in ethyl alcohol is a typical case and is illustrated in Figure 1. In the inset graph the dotted line represents an idealized curve for the interfering substances which together with the vitamin present give the absorption curve for the solution shown as a full line.

Readings, giving extinction coefficients denoted by the letters A, B, and C, respectively, are taken at wave lengths 325, 310, and 340 mμ—i.e., $\lambda_{\max.}$, $\lambda_{\max.} - 15$ mμ, and $\lambda_{\max.} + 15$ mμ. The absorption due to the pure vitamin A present at these three wave lengths is $A - (x + y)$ at 325 mμ, $B - (x + 2y)$ at 310 mμ, and $C - x$ at 340 mμ. For pure vitamin A alcohol in ethyl alcohol the extinction ratios E_{310}/E_{325} mμ and E_{340}/E_{325} mμ have been calculated in this laboratory as 0.846 and 0.771, respectively.

It follows therefore that:

$$B - (x + 2y) = 0.846\,[A - (x + y)] \qquad (1)$$

$$C - x = 0.771\,[A - (x + y)] \qquad (2)$$

Adding Equations 1 and 2 gives the expression

$$B + C - 2\,(x + y) = 1.617\,[A - (x + y)] \qquad (3)$$

which in terms of $x + y$, the irrelevant absorption at 325 mμ, reduces to

$$x + y = 2.60\,(B + C - 1.617\,A) \qquad (4)$$

The actual amount of this irrelevant absorption may conveniently be read directly from a graph as shown in Figure 1 and subtracted from reading A to give the extinction due to vitamin A. Alternatively, Equation 4 may be rewritten to give $A - (x + y)$, the extinction due to the vitamin A present, directly

$$A - (x + y) = 2.60\,[2A - (B + C)]$$

and taking $E^{1\%}_{1\,\text{cm.}}$ (325 mμ) for vitamin A alcohol in ethyl alcohol as 1780 this becomes

Concentration of vitamin A in test solution =
$$14.6\,[2A - (B + C)]\gamma/\text{ml.}$$

Equations similar to these but with new constants can be derived for other solvents and for the vitamin in esterified forms. Although a graphical solution could be applied to Morton and Stubbs's method, it is considered that the selection of wave lengths equidistant from $\lambda_{\max.}$ in the present method renders it simpler and therefore more readily applicable to routine work.

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Polarographic Behavior of Lead in the Presence of Gelatin

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MANY polarographic methods have been described for the determination of lead in various materials. In these methods, maxima of the waves must be suppressed, and gelatin is especially useful for this purpose. It has been pointed out (*2*) that gelatin in slight excess of that required to eliminate maxima greatly decreases the size of the wave. This phenomenon is discussed in detail in the present paper.

EXPERIMENTAL DETAILS

Solutions for polarographic measurements were made by transferring 10-ml. portions of a 2.5 millimolar solution of lead nitrate to 25-ml. volumetric flasks and, after addition of the materials necessary to make the proper supporting electrolytes, diluting to volume. All solutions were 0.1 *N* with respect to potassium chloride except as noted. The gelatin contents were adjusted by adding the required amounts of a freshly prepared gelatin solution to the flasks, and hydrochloric acid or potassium hydroxide was added to adjust the pH to the desired values. Measurements of pH were made with a Beckman meter, Model H-2, using the Type E (blue glass) electrode in the alkaline range. A silver wire was used as the anode in all solutions containing chloride. Otherwise the mercury pool was used. Oxygen was removed from the solutions with purified nitrogen. The drop times were observed after each measurement and the rate of flow of mercury from the electrode (in the solution) was checked several times during the working day. All measurements were made at 25° ± 0.1° C.

RESULTS

The results of the investigation are summarized in Figure 1, where the diffusion-current constants, calculated from the experimental data, are plotted as a function of pH for the three concentrations of gelatin used. In strongly acid solutions, the diminution of the waves is small, whereas in strongly alkaline solutions it is somewhat larger. In the pH range 3 to 13, the effect is very large.

Table I. Diffusion-Current Constant, *I*, of Lead in Various Supporting Electrolytes

Supporting Electrolyte	pH	*I*, Observed: In 0.01% gelatin	*I*, Observed: In 0.1% gelatin	*I*, Observed: In 1% gelatin	*I*, Literature (*3*) in 0.01% Gelatin
1 *N* sodium hydroxide	..	3.50	3.35	3.25	3.39
Alkaline tartrate	12.5	2.40	..	..	2.39
Neutral tartrate	6.6	2.31	1.86	1.63	2.30
Acid tartrate	4.6	2.42	1.30	0.94	2.37
0.1 *N* potassium chloride	5.1	3.85	3.55	1.65	3.80
20% citric acid	1.4	2.84	2.76	2.69	..
1 *N* hydrochloric acid	..	3.85	3.83	3.77	3.86
1 *N* nitric acid[a]	..	3.61	3.54	3.42	3.67

[a] Mercury pool anode used in measurements.

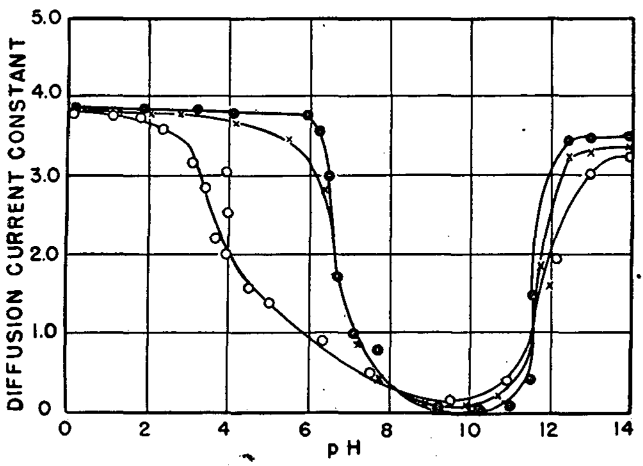

Figure 1. Diffusion-Current Constant for Lead as a Function of Gelatin Content and pH of Solution

● 0.01% gelatin × 0.1% gelatin ○ 1.0% gelatin

In the pH range 7 to 12, the diffusion current is very low and independent of the gelatin content. In this region, hydrolytic precipitation of the lead is probably the governing factor (*1*). Outside of this region, gelatin appears to remove lead from the solution in some manner. The behavior does not appear to be related to the viscosity of the solution.

In the region where diminution is appreciable, there is some variation of the wave height, depending upon the way in which the gelatin is prepared. Gelatin solutions prepared by soaking the material in cold water and then warming to effect solution showed a smaller effect on the waves than those prepared by heating nearly to boiling. Those prepared by the first method tended to appear turbid on dilution with water while the latter remained clear. The polarographic behavior supports the conclusion that the solution of the gelatin is more complete by the second method.

The diffusion-current constants found for lead in various supporting electrolytes are given in Table I. Here again, it is evident that the wave heights (diffusion-current constants) are greatly influenced by the gelatin content of the supporting electrolyte, except in very acid media. In all cases, except the alkaline-tartrate medium, the waves are well defined, even though in many cases the waves are diminished by the gelatin. In alkaline tartrate, the waves for the solutions containing 0.01% gelatin are poorly defined, in that they are spread out and never quite exhibit a diffusion current plateau. Addition of alkali to the supporting electrolyte improves the form of the wave, but even when 1.5 *N* in alkali the wave form was not all that could be desired. In the case of solutions with gelatin content 0.1% and greater, no acceptable waves were obtained in alkaline tartrate.

Methylcellulose has been recommended for suppression of maxima. The authors have used a product under the trade name Tylose with some success. Because of the difficulty in dispersing this material, they have tried sodium carboxymethylcellulose prepared by E. I. du Pont de Nemours & Company under the trade name Sodium CMC. This material is easily dispersed in cold water and is stable, so that stock solutions may be prepared and stored for future use. It was found that concentrations of the order of 0.01% of Sodium CMC are effective in suppressing maxima but, like gelatin, it diminishes wave heights when present in larger amounts. However, because of its stability, it is attractive as a suppressor and should be investigated more completely.

CONCLUSIONS

The results of this work emphasize that, when a choice is possible, strongly acid media are the most satisfactory for the polarographic determination of lead. When, because of the presence of interfering elements or other reasons, one of the other supporting electrolytes is required, the gelatin content of the solution must be controlled very carefully to avoid serious analytical errors.

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Crystalline Arsenates of Zirconium and Hafnium

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ANALYTICAL separations and determinations of elements in Groups III and IV of the periodic chart are often very difficult because of the formation of precipitates of poor quality. For example, the separation of zirconium from other positive ions by the precipitation of zirconium phosphate is often not nearly as effective as might be predicted on the basis of solubility differences. This is because it is very difficult to wash out occluded ions from a gelatinous, noncrystalline precipitate. Larsen, Fernelius, and Quill (*2*) have pointed out how the character of this precipitate can be improved. Willard and Freund (*3*) have further improved the process by forming the precipitating ion in the solution. Recently, it has been shown (*1*) that mandelic acid will quantitatively separate zirconium from most interfering ions.

Before the latter two publications appeared, the authors began an investigation to find if the separation of zirconium and hafnium could be improved by forming the arsenate ion in solution, through oxidation of arsenite. This paper shows the effects of varying conditions upon the character of the arsenate precipitate—in other words, shows how to get a good precipitate with zirconium which can be filtered and washed rapidly, rather than the common gelatinous kind which is so annoying.

EXPERIMENTAL

Experiments were carried out to determine the effect of varying the concentration of hydrochloric acid present, sulfuric acid present, zirconyl ions, and arsenite ions. The starting material was always air-dried zirconyl chloride or oven-dried (but not ignited) zirconyl hydroxide which was subsequently dissolved in hydrochloric acid. Nitric acid was employed as the oxidizing agent. Excess acid (usually hydrochloric) was used to prevent the formation of a basic precipitate.

Table I indicates that varying the concentration of hydrochloric acid had very little effect upon the crystal size and filterability of the arsenate precipitate. Although all these precipitates were good, those formed where the hydrochloric acid was only 0.6 *N* and 1.2 *N* were slightly better.

In another series of experiments, 11.77 grams of zirconium were used, and 14.9 grams of sodium arsenite, 30 ml. of concentrated hydrochloric acid, and 20 ml. of 6 *N* nitric acid were added. The volume of solution was 600 ml. at beginning of heating and 500 ml. after heating (Table II).

Table II shows that the concentration of sulfuric acid is of great importance. Although not listed in the table, the authors have determined that if no sulfate ions are present—i.e., if pure zirconyl chloride is employed—the zirconium arsenate precipitate is semicolloidal. Concentrations in the range of 1.2 to 1.8 *N* for sulfuric acid are evidently most satisfactory. Higher concentrations of sulfuric acid not only produced precipitates of poorer quality, but induced bumping of the solutions while they were being heated. The molar ratio of sulfate ion to zirconyl ion should always be kept above 1; in fact, the authors' experience indicates that the best results are secured when this ratio has a value of approximately 2 (note the excellent precipitate listed first in Table III).

Here we find the limits within which the concentration of zirconyl ion can be varied. If 600 ml. of solution contain 10 to 25 grams of zirconium, excellent results can be expected. Table III also rather clearly points out the solubility limits of zirconium arsenate under these conditions.

In a fourth series of experiments, 10.7 grams of zirconium were used, and 30 ml. of concentrated hydrochloric acid and 30 ml. of concentrated sulfuric acid were added. The volume of solution was 600 ml. at beginning of heating and 500 ml. after heating. In all cases the precipitate was crystalline and the solution filtered rapidly.

Table IV shows the effect of varying the concentration of the arsenite ions. Evidently within these limits the concentration of sodium arsenite is not important; all precipitations were rather satisfactory.

Table I. Effect of Hydrochloric Acid Concentration

Weight of zirconium, g.	11.77	11.77	11.77	11.77
Concentrated hydrochloric acid, ml.	30	60	120	240
Normality of solution in hydrochloric acid	0.6	1.2	2.4	4.8
Sodium arsenite added, g.	14.9	14.9	14.9	14.9
6 *N* nitric acid added, ml.	20	20	20	20
Concentrated sulfuric acid added, ml.	30	30	30	30
Volume of solution at beginning of heating, ml.	600	600	600	600
Volume after heating, ml.	500	500	500	500
Type of precipitate	Crystalline	Crystalline	Crystalline	Crystalline
Filtered	Rapidly	Rapidly	Rapidly	Rapidly
Time of settling, min.	2	2	2-3	3-4

Table II. Effect of Sulfuric Acid Concentration

Concentrated sulfuric acid added, ml.	10	20	30	60	100
Normality of solution in sulfuric acid	0.6	1.2	1.8	3.6	6.0
Type of precipitate	Small crystals	Crystalline	Crystalline	Semicolloidal	Colloidal
Filtered	Fairly well	Rapidly	Rapidly	Poorly	Very poorly
Time of settling, min.	10	2	2	Hours	Hours
Other remarks				Bumping	Bumping

Table III. Effect of Zirconyl Ion Concentration

Weight of zirconium, g.	23.54	11.77	5.9	3.9	2.9	1.7
Concentrated HCl, ml.	60	30	30	30	30	30
Sodium arsenite added, g.	29.8	14.9	7.6	5	3.7	3.7
6 *N* nitric acid added, ml.	40	20	10	7	5	5
Concentrated sulfuric acid added, ml.	30	30	30	30	30	30
Volume of solution at beginning of heating, ml.	600	600	600	600	600	600
Volume after heating, ml.	500	500	500	500	400	500
Type of precipitate	Crystalline	Crystalline	Crystalline	Crystalline	Cloudy	None
Filtered	Rapidly	Rapidly	Rapidly	Rapidly	..	..
Time of settling, min.	30 sec.	2	2-3	2-3	..	..

Table IV. Effect of Arsenite Concentrations

Sodium arsenite to precipitate	All Zr	$^2/_3$ Zr	$^1/_2$ Zr	$^1/_3$ Zr	$^1/_4$ Zr	$^1/_6$ Zr
6 *N* nitric acid added, ml.	35	20	17	10	8	5
Time heated over same type flame, min.	45	51	51	45	45	45

SUMMARY

A systematic study has been made of the factors involved in the precipitation of zirconium and hafnium arsenates by the formation of arsenate ion within the solution. If optimum conditions are employed, a precipitate of much better quality is obtained than the ordinary phosphate, hydroxide, or arsenate.

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RECEIVED May 31, 1949.

Colorimetric Determination of Cobalt with Nitroso R Salt in the Presence of Copper

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IT HAS been recognized that copper interferes in the colorimetric determination of cobalt with nitroso R salt, when the usual procedure is followed for small quantities of cobalt in metallurgical and similar products. Although McNaught (*3*) stated that copper up to 100 micrograms does not interfere with the determination of 1 microgram of cobalt, in his procedure the usual removal of copper with hydrogen sulfide is followed. In a later paper (*4*) he advocates the separation of copper when determining cobalt in animal tissues, but found that for plants and soils the separation was not necessary. Other investigators (*1*, *2*, *5*, *6*), presumably working with much larger quantities of cobalt and copper, have always removed the copper.

In this laboratory it has been found that by slightly modifying the procedure, quantities of cobalt in the range of 0.1 to 0.5 mg. can be determined in the presence of 10 to 12 mg. of copper.

REAGENTS

Nitroso R salt solution, 1 gram dissolved in water and made up to 500 ml.

Sodium acetate solution, 500 grams of sodium acetate trihydrate dissolved and made up to 1 liter with water.

Phosphoric-sulfuric acid mixtures, 150 ml. of concentrated phosphoric acid and 150 ml. of concentrated sulfuric acid, made up to 1 liter with water.

PROCEDURE

Take such a weight of sample, or aliquot after decomposition, that the quantity of cobalt present is not over 0.5 mg. and the copper does not exceed 10 to 12 mg. If this ratio of copper to cobalt is exceeded, remove copper by hydrogen sulfide or electrolysis. Decompose the sample with nitric and hydrochloric acids, with the addition of bromine for sulfides and hydrofluoric acid for silicates. Add 5 ml. of phosphoric-sulfuric acid, evaporate to strong fumes, and continue for 5 minutes. Cool, take up in 20 ml. of water, and boil. Add 30 ml. of sodium acetate solution, and 10 ml. of nitroso R salt solution, and boil vigorously for exactly 1 minute. Add 10 ml. of nitric acid to the boiling samples and if all the flocculent precipitate does not dissolve add 2 to 3 ml. more acid. Continue boiling for at least 1 minute, but do not prolong this operation beyond 2 minutes. Cool and dilute to 100 ml. with water. Filter through dry Whatman No. 40 papers and dry funnels into dry 250-ml. flasks. It will suffice to filter only 40 to 50 ml. Measure the absorption of the solutions in a photoelectric colorimeter in the usual way against a suitable blank and read the concentrations from a calibration curve prepared with measured amounts of cobalt (*6*).

DISCUSSION

If not more than 5 ml. of phosphoric-sulfuric acid are added to a 0.25-gram sample, it is only necessary to fume the latter strongly, and neutralization with sodium hydroxide is not required. Samples fumed to complete dryness and those having 4 ml. of phosphoric-sulfuric acid present gave the same results.

With 30 ml. of sodium acetate and 10 ml. of nitric acid, cobalt could be determined in the presence of 10 to 12 mg. of copper with little or no lowering of the result. With 23 to 25 mg. of copper the results were slightly low and were not rectified by the addition of more sodium acetate.

It is essential to boil the samples for 1 minute before the addition of nitric acid; otherwise the color of the cobalt complex will not be fully developed. The subsequent boiling of the acid addition is not so important, as vigorous boiling for 1 to 2 minutes will decolorize the iron complex, but it is not advisable to prolong this beyond 2 minutes.

In the authors' procedure the samples are not filtered until after the color has been developed. After the initial fuming, residual insoluble matter has no effect on the cobalt color, and the elimination of washing and evaporating solutions materially reduces the time for routine work.

Some typical results obtained with this procedure are given in Table I. The samples were those used in this laboratory as standards, and the values for "cobalt present" represent averages of closely agreeing results obtained by standard colorimetric, gravimetric, potentiometric, and electrolytic procedures for cobalt.

Table I. Recovery of Cobalt in Presence of Copper

Sample	Cu Present	Cu Added	Co Present	Co Found
	%	*Mg.*	%	%
Copper reverberatory slag	0.63	..	0.63	0.63
	0.63	11.5	0.63	0.63
	0.63	23	0.63	0.61
Electric furnace slag	0.71	..	2.27	2.27
	0.71	11.5	2.27	2.25
Copper converter slag	9.29	..	4.95	4.94

SUMMARY

By increasing the sodium acetate and nitric acid in the nitroso R salt procedure for cobalt, 0.1 to 0.5 mg. of the latter may be determined in the presence of 10 to 12 mg. of copper. This modification, together with the practice of filtering the sample only after the development of color at the end of the determination, results in a considerable saving of time.

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4,4'-Bis(dimethylamino)thiobenzophenone as a Reagent for Mercury and Certain Other Metals

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THIO-Michler's ketone, 4,4'-bis(dimethylamino)thiobenzophenone, has been found to form highly colored addition compounds with trace amounts of the ions of copper (cuprous), mercury (mercuric), silver, gold, platinum, and palladium, but not with those of other metals. Because of the high color intensity and chemical properties of these addition compounds, it has potential use for the detection and colorimetric determination of the various metals and in some cases as an internal indicator in volumetric analysis. Although its possibilities as an indicator in chemical analysis have been overlooked, a number of its reactions characterized by marked color changes have been reported (*1–4*).

4,4'-Bis(dimethylamino)thiobenzophenone is a purplish-red crystalline substance, nearly insoluble in water but readily soluble in a variety of organic solvents, to which it imparts a yellow color. It can be prepared from dimethylaniline by treatment with thiophosgene, by melting together tetramethyldiaminodiphenylmethane and sulfur, and from auramine by treatment with hydrogen sulfide. The latter method has been reported to be the best (*5*).

The material used in the work reported here was prepared at the Experimental Station, E. I. du Pont de Nemours & Company, Wilmington, Del. Analysis by T. M. Wooten and C. A. Rush, Analytical Branch, Chemical Division, Army Chemical Center, showed the following composition: calculated for $C_{17}H_{20}N_2S$, C 71.79; H 7.09; N 9.85; S 11.27. Found, C 71.9; H 7.1; N 9.8; S 10.9. Melting point 195° to 200° C. (dec.).

SENSITIVITY AS A DETECTION REAGENT FOR METALS

The minimum amounts of metals that can be detected visually in aqueous solution were determined by adding 1 drop of a 0.1% solution of 4,4'-bis(dimethylamino)thiobenzophenone in acetone to a known amount of the metal salt in 10 ml. of aqueous solution buffered with 1 ml. of 10% sodium acetate solution (the addition of sodium acetate is necessary because in the presence of free acids the reagent is oxidized to a blue substance by traces of chlorine that may be present in the water). The solutions were then viewed vertically in a 0.75-inch test tube and compared with a blank made up on the same manner. In the case of cupric acetate, the solution, after buffering with sodium acetate, was treated with a little hydroxylamine hydrochloride to reduce the copper to the cuprous form, which alone forms a colored complex with 4,4'-bis(dimethylamino)thiobenzophenone. Platinum solutions after buffering and treatment with sodium acetate were warmed to reduce the platinic to the platinous state. Palladium required an additional amount of acetone (1 ml.) to effect reduction to the palladous state, which took place under these conditions at ordinary temperature. The following sensitivities and colors were obtained:

	Sensitivity	Color
Hg^{++}	1 part in 5,000,000	Blue-green with red dichroism
Ag^{+}	1 part in 5,000,000	Red-purple
Cu^{+}	1 part in 1,000,000	Orange-pink
Au	1 part in 1,000,000	Blue-purple
Pt	Not determined	Red-purple
Pd	1 part in 10,000,000	Red-purple

The absorption spectra of the metallo complexes are shown in Figure 1.

No colors were obtained with aqueous solutions of the salts of arsenic, barium, bismuth, cadmium, cobalt, chromium(III), iron, manganese(II), nickel, osmium, rubidium, antimony, tin, strontium, thorium, thallium, uranium, zinc, or zirconium.

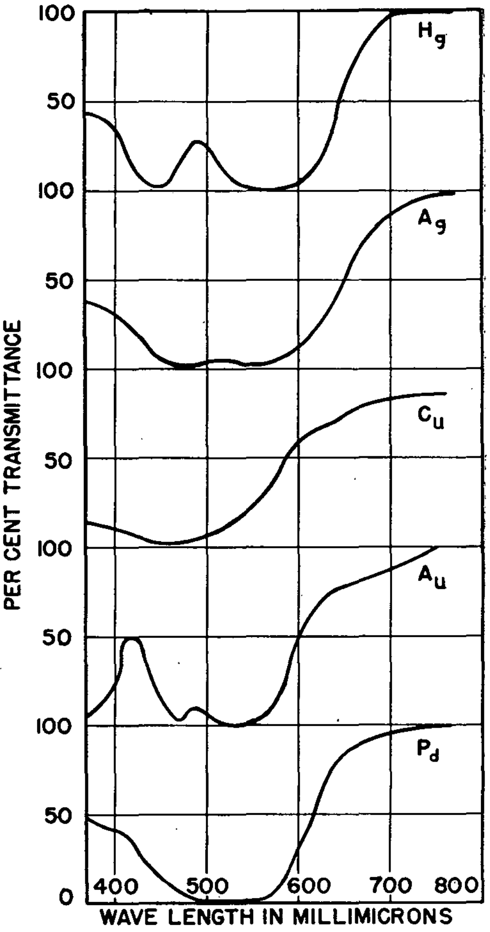

Figure 1. Absorption Spectra of Metallo Complexes of Thio-Michler's Ketone

Quantitative Colorimetric Analysis. As an excess of 4,4'-bis(dimethylamino)thiobenzophenone is necessary to convert the metal quantitatively into the colored complex and the reagent itself has a strong yellow color, the excess should be removed or destroyed before a color reading is made. In the case of mercury the excess can be extracted with ether, in which the blue-green mercuric complex is not soluble. To 10 ml. of the mercury solution and 0.2 ml. of a mixture of 1 part of glacial acetic acid and 2 parts of a 10% aqueous solution of sodium acetate in a 30-ml. separatory funnel, a 0.1% alcoholic solution was added drop by drop until the blue-green color first produced changed to a distinct reddish color [indicating excess of 4,4'-bis(dimethylamino)thiobenzophenone]; after 10 seconds 6 ml. of ethyl ether were added, the mixture was shaken thoroughly, and when the water and ether layers were completely separated, 5 ml. of the lower layer were drawn off into a calibrated Klett-Summerson colorimeter tube and a reading was taken immediately. The filter used was a No. 56, having a transmittance limit of 540 to 590 mμ which corresponds to the most characteristic absorption band of the mercury compound. Determinations of solutions containing 0.01, 0.05, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.5 p.p.m. gave a straight-line curve.

This quantitative colorimetric procedure is applicable only to mercury. It cannot be used for the other metals, as their compounds with 4,4'-bis(dimethylamino)thiobenzophenone show a varying degree of solubility in ether, the silver and copper complexes being the most soluble in this solvent. No work was done to develop procedures for the other metals.

Volumetric Analysis. In a preliminary study of the possible use of the reagent as an internal indicator in the volumetric determination of mercury by cyanide, or vice versa, 0.1, 0.01, and 0.001 molar solutions of mercuric nitrate and of potassium cyanide were buffered with sodium acetate and a few drops of a 0.1% solution of 4,4′-bis(dimethylamino)thiobenzophenone in acetone were added to them. When mercury solutions were titrated with cyanide the color change was from blue-green to pale yellow, the end point being very sharp with 0.1 and 0.01 molar solutions and satisfactorily sharp with 0.001 molar solutions. When cyanide solutions were titrated with mercury solutions, the color change was from pale orange to blue, with the same degree of sharpness as the reverse titration. In all cases the titrations were reproducible and the end points reversible. With accurately standardized solutions, a combining ratio of mercury to cyanide of 1 to 2 was found in either direction. The presence of chlorides did not interfere.

It was found that aqueous solutions of chlorides cannot be titrated with silver using 4,4′-bis(dimethylamino)thiobenzophenone as an indicator because its sensitivity is within the solubility range of silver chloride. However, chlorides in alcohol, acetone, or other solvents in which silver chloride is insoluble can be titrated with standard aqueous silver nitrate solution to a sharp end point.

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(2) Fehrman, W., *Ibid.*, **20**, 2857 (1887).
(3) Madelung, W., *J. prakt. Chem.*, **114**, 20 (1926).
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RECEIVED January 12, 1949. Presented before the Division of Analytical and Micro Chemistry at the 113th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill.

Control of Nitrite Interference in Colorimetric Determination of Phosphorus

ARNOLD E. GREENBERG, LEON W. WEINBERGER, AND CLAIR N. SAWYER
Massachusetts Institute of Technology, Cambridge, Mass.

IN THE colorimetric determination of phosphorus (*1*), involving reduction of ammonium phosphomolybdate by stannous chloride, nitrites have been found to interfere. Figure 1 shows the effect of various concentrations of nitrite nitrogen on the transmittance of light through a standard phosphorus solution using a wave length of 690 mμ. As the nitrite nitrogen concentration increases, the blue color rapidly fades and as little as 1 p.p.m. in the test sample introduces a considerable error in the determination.

In order to overcome this effect, various agents have been tried. An excess of stannous chloride (more than 0.25 ml.) will control limited concentrations of nitrites. However, as the intensity of the blue color formed is affected seriously by varying concentrations of stannous chloride, it does not seem desirable to use an unknown excess of this reagent.

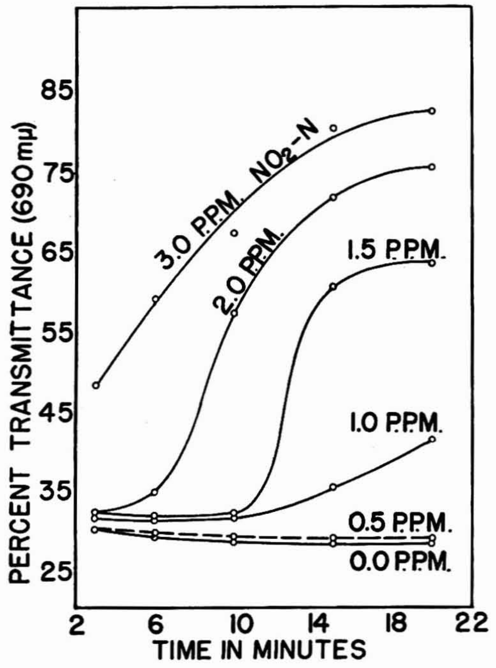


Figure 1. Effect of Nitrite Concentration on Transmittance in Phosphorus Determination

Table I. Control of Nitrite Interference in Determination of Phosphorus by Sulfamic Acid

	Light Transmittance, %, after 10 Minutes	
NO$_2$-N, P.P.M.	0.4 P.p.m. phosphorus	0.4 P.p.m. phosphorus plus molybdate-sulfamic reagent
0	27.6	29.6
2	26.7	29.9
5	65.0	29.4
10	87.8	29.0
15	87.0	27.6
20	88.6	29.5
25	89.0	30.4

Sodium azide when added to the sample in various concentrations controls nitrite interference, but it cannot be included as a component of the molybdate reagent because of the production and evolution of hydrazoic acid, a toxic and explosive gas.

Sulfamic acid was found to give good control of nitrite interference, as can be seen from Table I. It can be combined with the molybdate reagent. The sulfamic acid should first be dissolved in a minimum quantity of water and then added to a cooled sulfuric acid–molybdate solution. Ten grams of sulfamic acid per liter of reagent are sufficient to control concentrations of nitrites up to 25 p.p.m. Although the modified reagent does not appreciably affect light transmission by standard phosphorus solutions, for precise work a standard curve should be developed with the reagent. The reagent is stable for periods as long as 35 days.

LITERATURE CITED

(1) Am. Pub. Health Assoc., "Standard Methods for Examination of Water and Sewage," 9th ed., p. 80, 1946.

RECEIVED June 16, 1949.

Correction

In the article on "Differential Analysis with a Beckman Spectrophotometer" [Bastian, Robert, Webering, Richard, and Palilla, Frank, ANAL. CHEM., 22, 164 (1950)] in Figure 3 all the slit readings should be preceded by decimal points: 0.29, 0.24, 0.215, 0.158, and 0.13 mm.

CRYSTALLOGRAPHIC DATA

28. Vanillin I (3-Methoxy-4-hydroxybenzaldehyde)

Contributed by **WALTER C. McCRONE**, Armour Research Foundation, Illinois Institute of Technology,
Chicago 16, Ill.

VANILLIN can be crystallized from chloroform–carbon tetrachloride solutions in either of two polymorphic forms, I or II. When cooled slowly with stirring, pure form I, the stable form at room temperature, can be isolated. More rapid cooling of small volumes of solution gives mixtures of I and II. Because it requires some effort to obtain any form II and all normal recrystallizations would certainly yield form I, the properties on only this form are given below. Form II crystallizes from chloroform–carbon tetrachloride as rods and needles having α parallel to the length equal in refractive index to that of the Cargille index liquid 1.507 when that liquid is saturated with vanillin II.

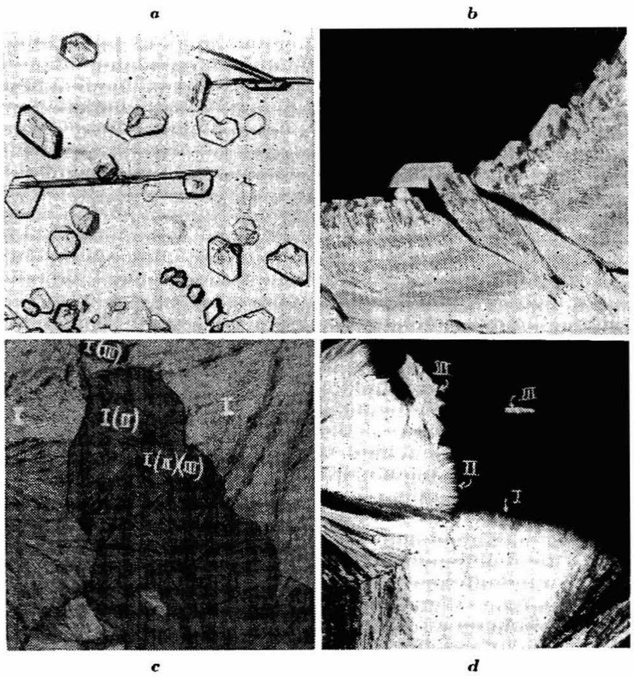


Figure 1. Vanillin

a. Vanillin I from thymol on microscope slide
b. Vanillin I during thymol mixed fusion
c. Vanillin I completely solidified, showing pseudomorphic regions
d. Vanillin I, II, and III crystallizing from melt

Two other polymorphs of vanillin are obtained only during fusion studies.

Crystal Morphology

Crystal System. Orthorhombic.

Form and Habit. Plates and tablets from chloroform lying on {100} showing bipyramids {111}; and prisms {120}. The commercial material is usually rods elongated parallel to *c*.

Axial Ratio. $a:b:c = 0.964:1:0.561$.

Interfacial Angles (Polar). $120 \wedge 1\bar{2}0 = 125°8'$; $011 \wedge 0\bar{1}1 = 58°38'$; $101 \wedge 10\bar{1} = 119°40'$.

X-Ray Diffraction Data

Cell Dimensions. $a = 7.88$ A.; $b = 14.04$ A.; $c = 13.53$ A.

Formula Weights per Cell. 8.

Formula Weight. 152.14.

Density. 1.34 (flotation); 1.36 (x-ray).

Principal Lines

d	I/I_1	*d*	I/I_1
6.79	1.00	3.11	Very weak
6.36	0.26	3.03	Very weak
6.09	0.32	2.94	Very weak
5.46	Very weak	2.84	Very weak
5.33	Very weak	2.71	Very weak
5.12	0.21	2.64	Very weak
4.85	Very weak	2.57	Very weak
4.56	Very weak	2.49	Very weak
4.41	Very weak	2.44	Very weak
4.20	Very weak	2.32	Very weak
4.07	Very weak	2.24	Very weak
3.93	0.47	2.19	Very weak
3.72	Very weak	2.13	Very weak
3.59	Very weak	2.10	Very weak
3.43	0.79	1.98	Very weak
3.31	1.00	1.89	Very weak
3.19	Very weak	1.85	Very weak

Optical Properties

Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.551 \pm 0.002$. $\beta = 1.694 \pm 0.002$. $\gamma = 1.80 \pm 0.005$.

Optic Axial Angle. (5893 A.; 25° C.). $2V = 75°$ (calcd. from α, β, γ).

Dispersion. $v > r$ (very strong).

Optic Axial Plane. 100.

Sign of Double Refraction. Negative.

Optical Orientation. $\alpha = c$; $\beta = a$; $\gamma = b$.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.679$. R (calcd.) = 38.7. R (obsd.) = 42.8.

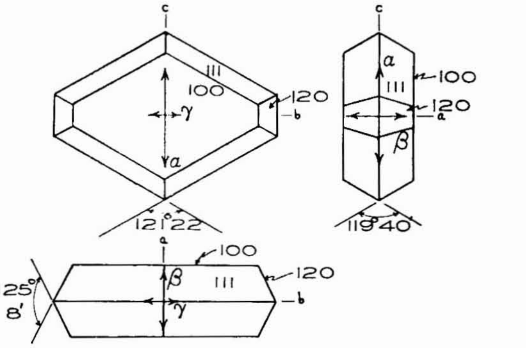


Figure 2. Orthographic Projection of Typical Crystal of Vanillin

Fusion Data. Spontaneous crystallization from the melt after complete fusion gives rise normally to three polymorphic forms (I, II, III) and may occasionally show a fourth (IV). The latter is very unstable and very rare; it is ignored in the following discussion. Vanillin I and II grow as spherulites, I more rapidly and finer grained than II. Both are more stable than vanillin III, which crystallizes spontaneously as small very slow-growing rectangles and aggregates of several rods. The rates of growth at room temperature are in the order I > II > III. The transition rates in the order III → I > II → I > III → II. The melting points are in the order I > II > III > IV.

A thymol mixed fusion shows typical profile angles of 121° 22' and 90°.

Contributions of crystallographic data for this section should be sent to Walter C. McCrone, supervisor, Analytical Section, Armour Research Foundation of the Illinois Institute of Technology, Chicago, Ill.

Correspondence

Short Procedure for the Determination of Sulfaquinoxaline in Feeds

Sir: The determination of sulfaquinoxaline in commercial feeds as described by Tyler, Brooke, and Baker [Anal. Chem., 21, 1091 (1949)] can be materially shortened by modifying the technique of preparing the extract.

Extraction. One gram of feed (0.75 to 1.5 mg. of sulfaquinoxaline) ground to pass a 1-mm. sieve is transferred to a 100-ml. glass-stoppered volumetric flask, and 25 ml. of boiling sodium hydroxide (5 ml. of 2.5 *N* sodium hydroxide in 1000 ml. of distilled water) are added.

The mixture is swirled to effect solution and diluted with 25 ml. of distilled water. Then 18 ml. of 15% trichloroacetic acid (15 grams per 100 ml.) are added, and the solution is diluted to volume with distilled water, shaken well, and allowed to stand for 5 minutes. It is filtered through an 11-cm. S. & S. 597 filter paper. The first 10 ml. are discarded and color is developed as described by Tyler, Brooke, and Baker.

The above changes do not affect the accuracy of the determination and reduce the time required to complete an analysis by one half.

S. W. Tyler
R. O. Brooke
W. S. Baker

Wirthmore Research Laboratory
Malden 48, Mass.

Book Reviews

A Basic Course in the Theory and Practice of Quantitative Chemical Analysis. *George L. Clark, Leonard K. Nash,* and *Robert B. Fisher.* xxii + 448 pages. W. B. Saunders & Co., Philadelphia, Pa., 1949. Price, $4.25.

This text is a rather brief one that is planned to cover the introductory year of quantitative chemical analysis. The authors, after two preliminary chapters, follow the order: gravimetric analysis, volumetric analysis, colorimetric analysis. Approximately 90% of the text, exclusive of appendixes, is devoted to conventional gravimetric and volumetric methods and the accompanying theory.

In comparison with other more or less similar texts, this one devotes relatively more space to volatilization methods, to the role of water of hydration or adsorbed water in quantitative work, and to the theory of precipitation. Three chapters are given to the last topic. The chief gravimetric determinations that are described in great detail are those of chloride, sulfate, and iron in simple mixtures. The section on separations and the analysis of limestone and brass is a very brief one, of only 14 pages.

In the volumetric section the preparation and use of standard solutions of the following substances are dealt with: acid, alkali, silver nitrate, ammonium thiocyanate, potassium permanganate, potassium dichromate, ceric sulfate, sodium thiosulfate, and iodine. A brief account of potentiometric titrations is included under the theory of oxidation-reduction titrations. The procedures given are for the most part those that are traditionally included in similar courses. An acidimetric procedure is, however, given for aluminum by the fluoride complex method.

The final section of the text proper consists of two chapters (22 pages). One chapter deals with colorimetry and touches on filter photometry and spectrophotometry. Procedures are given for pH measurement, determination of ammonia, and estimation of manganese in steel. The final chapter includes turbidimetry, nephelometry, electrodeposition with a procedure for copper, potentiometric titration, conductometric titration, polarography, emission spectroscopy, x-ray and electron diffraction methods, physical constants, and a selected bibliography. These topics receive only brief description in the ten pages that are allotted to them.

The appendixes deal with mathematical operations, standardization of weights, and statistical treatment of data, and tabular material is given on the e.m.f. series, solubility products, ionization and complex dissociation constants, specific volume of water at various temperatures, and atomic weights. Indicator solutions are tabulated in the final appendix, and the color intervals are shown inside the back cover. There is no table of logarithms.

Review questions or problems or both are provided for most of the chapters. The total number of problems is not large, nor are answers given for any of them. The type is rather small and the procedures are set in still finer print. The procedures are quite abbreviated. The authors and publishers have apparently done a very thorough job of proofreading. N. H. Furman

Introduction to Semimicro Qualitative Analysis. *C. H. Sorum.* ix + 196 pages. Prentice-Hall, Inc., 70 Fifth Ave., New York, N. Y., 1949. Price, $2.65.

This book is written as a text for a one-semester course, and according to its author was designed particularly for use in laboratory work accompanying the usual lecture coverage of the second semester of general chemistry. As might be expected of a text written for use in the first year of chemistry, little theory is included. The cation separations are made according to conventional schemes. Anions are classified by means of preliminary tests, and final evaluation is then made by interpretation of information gained during the cation analysis together with that obtained by certain "specific" tests for anions.

Some teachers will miss the use of preliminary exercises. A number of loose statements appear, such as the statement (page 16) that a normal solution of orthophosphoric acid contains one third the molecular weight of the acid per liter; on the next page the weight of salt to be used to make 1 liter of normal solution is determined by dividing the molecular weight of the salt by the total valence of the negative radical. Unqualified statements such as the one declaring that all metal chlorides are soluble except those of silver, mercury I, and lead may avoid confusing students but are somewhat disturbing. The above criticisms are not to be construed as condemning this book—many teachers prefer to avoid certain exceptional cases and confusing details.

The laboratory directions seem clear and reliable. The confirmatory tests are especially good: in a number of cases organic reagents are used. Throughout the book the author has made liberal use of explanatory notes and equations are presented for most of the important reactions studied. Philip W. West

A.S.T.M. Standards on Textile Materials (with Related Information). *A.S.T.M. Committee D-13 on Textile Materials.* xii + 574 pages. American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pa., 1949. Price, $4.50 (paper).

The 341 members of Committee D-13, through their 21 subcommittees, have guarded well the high standard for standards maintained by the A.S.T.M. Their care and skill are manifest in the 51 standards and 33 tentative standards (cotton 23, wool 11, rayon and silk 9, asbestos 7, glass 6, bast and leaf fibers 5, general and test methods 23) for 1949.

Accessory features are presented in three glossaries, eleven appendixes, three papers on x-ray studies, and several lists of publications and of committee officers or members. These features rank high in utility as supporting material for the standards and even as reference sources on their own.

The three glossaries appended to D 123–49 (definitions of terms) constitute a solid base for mutual understanding through use of terms in their accepted meanings. It is inconvenient that there are twelve separate alphabetic sequences in D 123–49 and its appendixes; an index would be helpful as a sort of "basic English" for textile technology.

Any one who still thinks "A.S.T.M. Standards on Textile Materials" is a book solely for those who make and use textile specifications should take a second look. Liberal citation of supporting literature throughout the text and tables makes the volume a reference tool for textile technologists in many interests other than standards.

JULIAN F. SMITH

Standard Methods for Testing Petroleum and Its Products. 10th ed. lvi + 660 pages. Institute of Petroleum, 26 Portland Place, London, W. 1., 1949. Price, 31s. 6d.

This is the tenth edition of the British counterpart of Committee D–2's "A.S.T.M. Standards on Petroleum Products." The volume under review contains 116 tests, compared with 125 in that of D–2; the I.P. publication lives up to its title by covering substantially the whole field, whereas A.S.T.M. tests for asphaltic products and knock characteristics are published elsewhere than in the D–2 report.

Some of the I.P. tests are less comprehensive than the A.S.T.M. methods—for example, I.P. 111/49(T) and 110/47T cover the determination of calcium and barium, respectively, in lubricating oil; these are included in D 811–48, "Chemical Analysis for Metals in Lubricating Oils."

It is not to be supposed, however, that A.S.T.M. publications contain everything in "Standard Methods." A.S.T.M. D 285–41, "Distillation of Crude Petroleum," is confined to determining the percentage and distillation range of the naphtha. "Standard Methods" offers three tests: one covering the light constituents by atmospheric distillation, one examining the residue by vacuum distillation, and one separating the crude into 2% fractions through a fractionating column, using both atmospheric and reduced pressures.

A particularly valuable feature of "Standard Methods" is Appendix A, a boiling point conversion chart correcting, to 760 mm., observations made from 0.01 to 100,000 mm.

Figures and printing are excellent. In addition to the general index, there are numerical and alphabetical lists of the methods. The tests are printed in alphabetical order, which makes it decidedly easier to find the desired method without reference to the index.

Those concerned with a testing of petroleum products for export will need this volume, and anyone who frequently refers to D–2 will find "Standard Methods" a highly valuable additional source of reliable information.

S. W. FERRIS

Pittsburgh Conference on Analytical Chemistry

THE Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, sponsored by the Analytical Division of the Pittsburgh Section, AMERICAN CHEMICAL SOCIETY, and the Spectroscopy Society of Pittsburgh, was held in Pittsburgh February 15 to 17, 1950. Abstracts of the papers presented are given here.

Fundamental Operations of Analytical Chemistry. PHILIP J. ELVING, The Pennsylvania State College, State College, Pa.

The basic stages in the analytical process were discussed with emphasis on the acute problems of isolating the material in a state suitable for measurement of the desired component and of making the measurement itself. The former problem was considered on the basis of the general techniques available for separating and segregating substances by chemical and physical methods. The process of measurement as applied to chemical substances was considered on the basis of the discriminatory power of the type of measurement used—i.e., on the ability of the measurement to distinguish between different substances. The fundamental principles of physical measurement as used in analysis were outlined.

Progress in Spectrographic Instrumentation. GEORGE R. HARRISON, Massachusetts Institute of Technology, Cambridge, Mass.

The paper dealt with the greater efficiency of the echelle grating spectrograph. The chief advantage of this relatively new technique is the improved resolution and the ease by which complex spectra can be recorded on a relatively small plate instead of several as required with the large prism or grating-type spectrographs. A paper has been published on this subject by the author [*J. Optical Soc. Am.*, 39, 522 (1949)]. The preparation of efficient echelles has yet to be worked out, but a paper read by David Richardson, R. S. Wiley, and C. J. Sheldon, Bausch & Lomb Optical Co., at the Optical Society of America meeting in New York, March 9 to 11, 1950, indicates that progress is being made.

The Industrial Analytical Chemist in a Changing Scene. WILLIAM SEAMAN, American Cyanamid Co., Calco Chemical Division, Bound Brook, N. J.

It is recognized among analytical chemists that the practice of analytical chemistry, especially in industry, has changed markedly in recent years, and is still in process of evolution. In this talk an attempt was made to trace some of the causes and to define some of the elements of this change. Consideration was given to some of the problems raised by this state of affairs—for example, what is the most fruitful approach of the analytical chemist to his responsibilities in an industrial organization, particularly in process control and development? There was also discussion of the kind of training and experience desirable for the analytical chemist to be able to play his part most adequately in the changing analytical scene, and of what industry might do to help assure itself of a supply of analytical chemists who could adequately meet the demands of modern industrial analytical chemistry.

Spectrochemistry at the Bureau of Mines in Ottawa. J. CONVEY, Department of Minerals and Natural Resources, Bureau of Mines, Ottawa, Ontario, Canada.

A very brief presentation of the spectrographic facilities of the Bureau of Mines in Ottawa was given, and the nature of the routine requirements for these services was described. Representative examples of current spectrochemical investigations were discussed—for example, the determination of magnesium in ductile cast iron, high purity and alloy zinc analyses, and mineral analyses.

An Analysis of Analyses. CHESTER M. ALTER, Boston University, Boston, Mass.

That the study and practice of the science of chemistry have been important contributing factors in our national economy cannot be denied. Much has been written concerning the economic aspects of our science. However, practically all such writings pertain to research rather than to analysis for control purposes. For instance, one can find estimates of how much is being spent for research but nothing about that spent for analysis and measurement.

Analytical control of raw materials, processes, and product, usually carried out in plant laboratories under the jurisdiction of production or plant managers, constitutes an important production cost in most industrial operations and in some it is a substantial cost. One can hardly imagine a commercial product which has not had its chemical composition and physical properties determined and recorded. This record of its composition and properties is the product's pedigree, the pride of the producer and the hope of the purchaser.

The diversity of modern raw materials and the complexity of modern industrial processes have made necessary modern methods of analytical control. Production management must be alert to economic values which can result from adoption of rapid analytical methods.

This paper presented a case study of the analytical control of raw material, process, and product of a limited number of companies of diversified types.

Although the authors of most textbooks in analytical chemistry would deny they have written their books for use primarily in teaching analytical methods and procedures of industrial importance, one wonders why such determinations as calcium and magnesium in limestone, iron in iron ore, copper in brass, etc., are described in practically all quantitative analysis textbooks. An analysis of analyses performed in industry or in pure scientific research gives a result which does not closely check the training given in many college courses in analytical chemistry.

As long as the composition and properties of materials are important in our industrial economy, so long will their determination be important to those who make and use these materials. This important work needs, and is increasingly getting, top-level attention.

Recent Developments in Infrared Spectrometry. VAN ZANDT WILLIAMS, The Perkin-Elmer Corp., Glenbrook, Conn.

World War II saw infrared develop from an academic pursuit into a standard research and analytical technique. In the subsequent period the field has widened tremendously from the war-established base.

In instrumentation, the development of fast heat detectors has permitted chopped beam radiation as well as excellent ratio photometry. With the advent of an infrared spectrophotometer with its possibilities of solvent compensation, the infrared sampling problem becomes easier and more standardized results can be obtained.

In application, straightforward analytical techniques have been improved, so that standardized procedures are being published on a scale comparable with that of visible colorimetry. An equally important development is the application of the method to qualitative organic analysis. This latter work has become particularly prominent in the biochemical and pharmaceutical fields.

Titrations with Ethylenediaminetetraacetic Acid. HARVEY DIEHL, Iowa State College, Ames, Iowa.

A group of polybasic acids, of which ethylenediaminetetraacetic acid, $(HO_2CCH_2)_2NC_2H_4N(CH_2CO_2H)_2$, is typical, have the property of forming remarkably stable, complex ions with the alkaline earth and other bivalent metals. The materials dissolve even the most insoluble calcium compounds, calcium oxalate, bone, egg shell, and milk stone, for example. It is possible to use the sodium salt of ethylenediaminetetraacetic acid as a standard solution for the titration of calcium. It is necessary to control the pH of the solution. Various methods are available for locating the equivalence point. A number of metals interfere with the titration, but it can be used to advantage to determine the calcium plus magnesium in hard waters, extracts of soils, and pure sodium and potassium compounds. Most anions are without effect and the titration is successful in the presence of large amounts of alkali metal salts. By combining this titration with colorimetric methods for magnesium and calcium, the total hardness, calcium, and magnesium in waters can be readily evaluated.

Determination of Fluoride. HOBART H. WILLARD, University of Michigan, Ann Arbor, Mich.

Fluoride is usually separated by the Willard and Winter method from the many substances which interfere with its determination. The fluoride is steam distilled as hydrofluosilicic acid at 135° to 150° C. from a perchloric or sulfuric acid solution.

Colorimetric methods usually depend upon the formation of stable complexes with aluminum, ferric iron, titanium, or zirconium, thereby bleaching to a certain extent colored compounds of these elements, such as ferric thiocyanate, peroxytitanic acid, and zirconium alizarine lake.

Gravimetric methods involve weighing calcium fluoride, lead chlorofluoride, triphenyltin fluoride, rare earth fluorides, and a few others. The lead chlorofluoride is most satisfactory for moderate or large amounts because of its dense, crystalline nature.

Volumetric methods involve the direct titration of fluoride by thorium, cerium, or yttrium or the formation of complexes with aluminum, ferric iron, or zirconium. Titration with thorium nitrate is most commonly used. At the equivalence point the thorium forms a pink to magenta lake with alizarine red S. The end point is readily distinguished by some operators but to many others it is indistinct. Many conditions, such as pH, must be carefully controlled.

With the able assistance of Charles A. Horton an extensive investigation has been carried out at the University of Michigan to find a better indicator for the thorium titration. More than 300 different compounds were tested by both visible and ultraviolet light and five were found fairly satisfactory. Quercetin and morin are the best, either as one-color indicators in ordinary light or as fluorescent indicators. A photofluorometric titration was developed in which the titration cell is placed in a fluorometer and a titration curve obtained by plotting the readings against the volume of thorium nitrate solution added and extrapolating the linear portion of the curve before and after the end point. The range is from 0.1 to 15 mg.

Another method depends upon the decrease in the fluorescence of the aluminum-morin lake as the amount of fluoride increases with the formation of aluminum fluoride complexes. This is satisfactory for amounts of fluoride less than 20 micrograms.

Electrode Problems in Electrometric Measurements. GEORGE A. PERLEY, Leeds & Northrup Co., Philadelphia, Pa.

Factors governing the selection of electrode systems were discussed. Liquid junction devices (salt bridges) were considered and the errors of several commercial types were contrasted. These hidden errors have generally been overlooked or erroneously attributed to the measuring electrode. The importance of the proper preparation of a saturated calomel half-cell was stressed particularly in reference to the temperature-hysteresis errors of the calomel reference electrode. A master electrode system and standard reference buffers as a means of periodically checking operating electrode systems, junction errors, etc., were suggested as essential for all laboratories concerned with electrometric measurements.

Determination of Nitric Oxide and Nitrogen Dioxide in Admixture. E. ST. CLAIR GANTZ, GERALD C. WHITNACK, CLIFFORD J. HOLFORD, AND G. B. L. SMITH, U. S. Naval Ordnance Test Station, China Lake, Calif.

The nitrometer method of analysis and the permanganate titration of nitrosylsulfuric acid have been investigated as a combined analytical method for the determination of nitric oxide and nitrogen dioxide in admixture. The method depends upon the absorption of the gases in 95% sulfuric acid, forming nitric acid and nitrosylsulfuric acid. Total nitrogen in the absorption medium is determined by a nitrometer analysis and the nitrous nitrogen is determined by a permanganate titration.

Samples of pure nitric oxide and pure nitrogen tetroxide were used in determining the precision of each method alone and combined. With pure nitrogen tetroxide precisions corresponding to 100.2% recovery for the nitrometer method and 100.4% recovery for the permanganate titration were established. For samples of liquid nitrogen tetroxide containing 1 to 15% nitric oxide the precision of the combined method corresponds to 100.2% recovery of nitrogen dioxide and 98 to 99% recovery of nitric oxide.

A gas absorption apparatus, with a special chamber for breaking ampoules of liquid nitrogen tetroxide in an air and water-free system, was described in detail.

Colorimetry Applied to High Absorbance Systems. C. F. HISKEY, Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

Two prime requirements must be met if precision colorimetric measurements are to be made with a photoelectric colorimeter: The colorimeter must have sensitivity in transmittance measurements—i.e., the uncertainty of reading the transmittance scale $\Delta\ (I_1/I_0)$, must be as small as practicable. The absorbance, $(A)_1$, of the solution should be large.

Of these two requirements the former is the more important, but once the sensitivity is fixed the analyst has only the latter variable to adjust. In this discussion the case of high absorbance colorimetry was emphasized, because this is a relatively undeveloped area of measurement.

High absorbance colorimetry is treated as a measurement of the relative transmittance of an unknown, compared not to the solvent but to a standard solution. The absorption law expression then becomes

$$I_2/I_1 = 10^{-abc_1(\alpha - 1)}$$

with $\alpha = c_2/c_1$ and with I_2 and I_1 equal to the intensities of the light beam getting through solutions of concentration c_2 and c_1, respectively.

If we now derive a function for the relative error involved in measuring c_2, this becomes

$$\frac{\Delta c_2}{c_2} = \frac{\Delta(I_2/I_1)}{\alpha A_1[e^{-A_1}(\alpha - 1)]}$$

and thus the effect of the absorbance of the standard and of the concentration ratio on the precision may be evaluated. Graphical plots of this function were discussed in some detail, but one of the major consequences is that above an absorbance of 0.4343 the precision increases directly with the absorbance when α or I_2/I_1 approaches unity. This means that for any particular instrument which the analyst may have, the precision is limited in part by the intrinsic length of its response scale. This length may be defined as $L = 10A_{\max}$ where $A_{\max}$ is the highest absorbance for which the relative transmittance scale can be set to unity on any particular instrument. Because this is an over-all response scale, its characteristics are controlled by all the factors which affect the total response of the instrument—i.e., the light source, instrumental optics and the photo cell and its accompanying electrical network, etc. Because some of these factors are affected by the wave length of the light being used, it is to be expected that the ultimate precision attainable is very much a function of the wave length used. Data for some commercial instruments were presented to illustrate this fact.

To achieve chemical analyses with a precision of 1 part in a 1000 or better, the analyst must give careful consideration to a number of factors which he has tended to ignore. Among the more important are:

Constancy among the cuvettes with respect to the length of the light path. The ratio of two cell thicknesses β, defined as b_2/b_1, must be evaluated with the maximum precision attainable with the instrument. The modified relative transmittance becomes $I_2/I_1 = 10^{-ab_1c_1(\alpha\beta - 1)}$ in order to accommodate the length correction term.

The necessity of using colored ions or molecules possessing sufficient stability so that fading or enhancing effects with time, temperature, or electrolyte concentration are sufficiently controlled. Where these cannot be controlled properly a new color reaction must be devised.

Reflectances at interfaces are affected if the refractive index varies among the several solutions being analyzed. While this correction is usually small, it should not be assumed that it can always be neglected.

Apparent deviations from the absorption law. These deviations are of a positive or negative character and lead to an enhancement or a diminution of the precision, respectively. A treatment of the precision function in these cases was given.

Illustrations of the over-all analytical precision which may be achieved in a number of cases were presented with the necessary chemical details.

Some Unusual Cases in Photometric Analysis. H. A. LIEBHAFSKY, General Electric Co., Schenectady, N. Y.

Over the years, several unusual cases in photometric analysis have been encountered in this laboratory.

1. Pronounced deviations from the additivity relationship normally valid for the x-ray absorption of binary mixtures.
2. Spectrophotometric determination of cations in suspensions of their hydroxyanthraquinone lakes.
3. Spectrophotometric evaluation of spot tests.
4. X-ray determination of the rate of pit growth in stainless steel.
5. Use of a light meter (or exposure meter) in photometric analysis.

Each of these cases was briefly discussed in light of Beer's original experimental work and Beer's law as usually treated today.

Ultraviolet Spectrophotometric Determination of Molybdenum. GEORGE TELEP AND D. F. BOLTZ, Wayne University, Detroit, Mich.

A study of the absorption spectra of peroxymolybdic acid has been made for the purpose of determining the effect of acidity, concentration of hydrogen peroxide, and various diverse ions. Conformity to Beer's law was found at 330 and 274 millimicrons under certain conditions. The nature and elimination of several interferences were discussed. The proposed procedure is applicable to the determination of 0 to 150 p.p.m. of molybdenum.

Simultaneous Determination of Cobalt, Copper, and Iron. R. E. KITSON, E. I. du Pont de Nemours & Co., Inc., Polychemicals Department, Wilmington, Del.

A rapid method has been developed for the simultaneous determination of cobalt, copper, and iron. In solutions containing 50% acetone and an excess of thiocyanate ion, cupric ion develops a red-brown color, ferric ion the characteristic blood-red color, and cobaltous ion a deep blue color. Under proper conditions, all three colors can be developed quantitatively in the same solution. Absorption measurements on the solution at 380 mμ (copper), 480 mμ (iron), and 625 mμ (cobalt) permit the calculation of the amounts of each constituent present. As little as 50 micrograms of copper or cobalt or 5 micrograms of iron can be determined by this method. The color development is no more complicated than for a standard single component method, and the calculations are only slightly more involved. The optimum conditions for color development and the interferences of about 50 diverse ions have been studied. No serious interferences have been found.

If cobalt alone is to be determined on the sample, the procedure is modified slightly to reduce the ferric and cupric ions with stannous chloride, and the determination is made in standard single-component fashion. The method is not recommended for the determination of copper alone or the determination of traces of copper in the presence of iron and/or cobalt. The method has been used with success on a wide variety of samples over the past two years.

Behavior of Nitrogen and Some of Its Compounds in Steel. H. F. BEEGHLY, Jones and Laughlin Steel Corp., Pittsburgh, Pa.

Experimental work with the ester-halogen reagents, first used in determining aluminum nitride in steel, was outlined. The influence of thermal treatment and some of the elements commonly present in steel on the formation of certain acid-soluble compounds in steel was considered on the basis of analytical data obtained with these reagents supplemented by spectroscopic and x-ray diffraction techniques.

Use of Sensitive Reagents for Semiquantitative Analysis. P. E. WENGER, D. MONNIER, AND Y. RUSCONI, University of Geneva, Geneva, Switzerland (translated and presented by HENRY FREISER).

A simple, rapid method for semiquantitative analysis based on the determination of the limiting concentrations (grams per liter) in which a reaction is visible in a standard and in an unknown solution was described. A comparison of these limiting concentrations is the basis for calculating the per cent of desired constituent. Application of the method to the analysis of chromium and iron was described. Errors of the order of 10 to 20% on iron samples containing 0.003 to 1.5% iron are predicted. This method has the advantage of economy over those requiring instruments.

Determination of Organic Functionality by Molecular Spectroscopy. NORMAN D. COGGESHALL, Gulf Research and Development Co., Pittsburgh, Pa.

Both Raman and infrared absorption spectroscopy yield fundamental information which may be correlated with the vibrational modes of a molecule. The vibrational modes may be classified in two categories: skeletal modes and modes due to specific atomic groupings. The former vary with molecular structure and furnish a unique set for each molecule which makes possible the analytical applications. The latter are essentially invariant between different molecular species and hence furnish the basis for molecular structure determinations. At present most of the applications of infrared and Raman spectroscopy to analytical chemistry may be classified as: (*a*) analysis of gases obeying Beer's law, (*b*) analysis of gases exhibiting pressure broadening, (*c*) analysis of unassociated liquids, (*d*) analysis of liquids subject to intermolecular association, (*e*) group type analysis, and (*f*) molecular structure determination. The principal aspects and some detail cases of each were discussed. Items (*a*) and (*c*) are in a fairly satisfactory state of development; (*b*), (*e*), and (*f*) have been very profitably applied yet are far from being completely developed; whereas a great deal of research and development work is yet to be done on (*d*).

When photons are absorbed in the ultraviolet region of the spectrum there is an excitation of the electronic energy levels.

These are a function of the valence bonding electrons. In general, absorption in the ultraviolet is due to the resonance characteristics of atomic groupings called chromophores. The chromophores are atomic groupings, usually possessing unsaturation, whose first excited electronic energy levels are low enough to be excited by ultraviolet light. Examples of chromophores are the phenyl ring, the carbonyl group, the naphthalene structure, etc. Conjugation between chromophores allows a resonance interaction with the consequent appearance of new absorption bands. This is of great value in molecular structure studies and in studies of steric hindrance. In the absence of such conjugation or other similar effects, the absorption by a chromophore is independent of the remainder of the molecule. Thus, materials absorb in the ultraviolet according to class. This is both an advantage and a disadvantage. It allows the determination of chromophoric materials in the presence of nonabsorbing materials. However, the multicomponent analysis of mixtures of materials of the same chromophoric class is often impractical because of the similarities of spectra. There are several important exceptions to this. These and other typical applications were discussed.

The rotational energy levels of certain polar molecules may be observed by microwave absorption. Although this offers a new tool, there is some question as to the extent of its ultimate application to analytical chemistry. Some of the limitations of the method are fundamental in the phenomena utilized. Some discussion of the technique and its scope and limitations was given. Although the mass spectrometer does not utilize the absorption of radiant energy, it is closely allied to the above techniques in many ways. In this instrument the ionization fragments resulting from the electron bombardment of molecules are sorted and their abundances plotted as a function of mass. The differences in the mass spectra between unlike molecules allow important analytical applications to be made. The scope and limitations of these were discussed.

Determination of Low Concentrations of Low Molecular Weight Solutes in Nonpolar Solvents. M. R. Meeks, V. E. Whittier, and C. W. Young, Dow Chemical Co., Midland, Mich.

At concentrations of 0.1% or less of low molecular weight polar solutes in nonpolar solvents it is found experimentally rather difficult to carry out meaningful analysis because of accidental losses of solute. The reasons behind the difficulties encountered were discussed in terms of well-established principles of the dilute solution and pointed up the necessary precautions to be taken.

Applications of a Double-Beam Infrared Spectrophotometer to Chemical Problems. John U. White, The Perkin-Elmer Corp., Glenbrook, Conn.

The value of a versatile double-beam recording infrared spectrophotometer in solving chemical problems was illustrated with several examples. Reproductions of original records showed the advantages resulting from high speed, resolution, stability and flexibility in the measurement of spectra, analysis of samples, and the determination of structure. The possibility of compensating for the scattering losses in mulls and for the solvent absorption in solute study is particularly useful.

Application of Infrared Gas Analyzers. D. D. Friel, E. I. du Pont de Nemours & Co., Inc., Engineering Department, Wilmington, Del.

The self-filtering infrared gas analyzer is the first almost universal gas analyzer available for the continuous analysis and control of plant gas streams. Its application to complex gas mixtures under varying plant conditions has been extended by a number of recent developments.

The application of these instruments to a variety of typical and special plant problems was discussed, and the practical solutions to a number of problems encountered in their use were described.

Infrared Analysis of Certain C_1-C_4 Hydrocarbon Mixtures. James D. Stroupe, Rohm & Haas Co., Philadelphia, Pa.

Infrared analytical methods are developed for the direct determination of small amounts of ethane, propane, *n*-butane, and isobutane in natural and purified methane streams. Accuracies of $\pm 0.1\%$ are obtained with the 27-cm. gas absorption cell of the Beckman IR-2 spectrophotometer. An additional comparison cell set permits the direct determination of methane to within $\pm 0.5\%$. The methods have proved satisfactory in routine application for the past 2 years.

Triethylamine Addition as a Means of Increasing the Applicability of Infrared-Transmitting Solvents. J. S. Ard and Thomas D. Fontaine, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Beltsville, Md.

The scarcity of solvents suitable for infrared work is an acute problem. The two solvents considered to be the best for infrared investigations—carbon disulfide and carbon tetrachloride—have been modified by the addition of 0.5 to 2.5% triethylamine. The resulting mixtures transmit sufficiently for easy use in all the regions from 2 to 15 microns except those already obliterated by the unmodified solvents. Some acids for which no other suitably transmitting solvent could be found readily dissolved in these mixtures. The carbon disulfide–triethylamine mixture is sufficiently stable for use after several days, whereas the carbon tetrachloride–triethylamine mixture is sufficiently stable only for quick use (1 to 2 hours).

Quantitative Infrared Analysis of Solid Isomeric Mixtures. H. G. Montieth, Aluminum Research Laboratories, New Kensington, Pa.

A method has been developed for determining the isomer present in a two- or three-component solid mixture by infrared absorption measurements. The compounds studied were derived from products of a Diels-Alder condensation, and consisted of a cyclohexene ring with two substituents ortho, meta, or para to each other.

For measuring the infrared absorption characteristics of a solid, the technique of preparing the compound by grinding a sample to a powder and mulling with mineral oil was used. The absorption spectra of the pure isomers were first measured with the paste pressed between two rock salt plates. With these spectra as standards, the absorption of a mixture of solid isomers was measured over the desired wave-length range. The easiest method of attack is to repeat the sample thickness as closely as possible by identical methods of preparation. The analyses can then be made for component ratio by measurements of relative band depths or by including a known amount of some new material as an internal standard. An example of this type of analysis was given.

The adduct of isoprene and acrylonitrile, a mixture of 1,2,5,6-tetrahydro-*p*-tolunitrile and 1,2,5,6-tetrahydro-*m*-tolunitrile, was converted to the corresponding amides, and infrared analysis of the latter mixture gave 93% para and 9% meta isomer. These are mean values of several determinations, the average deviation being less than 2. Results were in satisfactory agreement with the percentages determined by fractional crystallization.

Infrared Analysis of *cis*- and *trans*-Decalin. J. Seidman, Sinclair Refining Co., Harvey, Ill.

A solution containing a mixture of *cis*- and *trans*-Decalin along with other noninterfering components can be analyzed so as to give the percentage of Decalin present as a sum of the percentage of cis- and trans-isomers present. The method used is that of determining the absorbance, A, of either *cis*- or *trans*-Decalin and then reading the representative percentage of the component present from a working curve. The baseline technique is utilized so as to eliminate, as much as possible, errors which would be introduced by the interference of other compounds. The empolyment of absorptivities is not satisfactory because of deviations from the Lambert-Beer law. The results check, by a weight per cent analysis, within experimental accuracy.

Infrared Spectra of Phenols. R. A. Friedel, Office of Synthetic Liquid Fuels, U. S. Bureau of Mines, Bruceton, Pa.

Infrared spectra were presented for 22 phenols, principally the simple alkyl derivatives. The well-known steric hindrance effect of ortho-substituents on the OH band is demonstrated. On the basis of the OH bands at concentrations of 4.70 mole % in carbon disulfide, the phenols are divided into four main classes: no ortho-substituents, one methyl group in the ortho position, larger than methyl group in the ortho position, and two methyl groups in both ortho positions. These classes differ as to band position or absorption intensity or both; intensities within a class are constant to within $\pm 3\%$ absorption.

An outline of the quantitative method for analysis of the 13 isomers of C_6, C_7, and C_8 phenols was presented.

Quantitative Analysis with Double-Beam Infrared Spectrophotometers. David Z. Robinson, Baird Associates, Inc., Cambridge, Mass.

There are various factors affecting the accuracy of quantitative analysis with both single- and double-beam spectrometers. In-correct reading of the 100% line, stray light effect, and transmission value produce different errors. When the width of the slit is greater than the width of the absorption band used, deviations from Beer's law may be expected. The effect of these deviations on the accuracy of analysis was discussed. It was shown that when Beer's law does not hold, the law of additivity of optical density will not be applicable.

Double- and single-beam infrared spectrophotometers were compared for use in quantitative analysis. Possibility of making direct determination of the true 100% line and of eliminating the spectra of one or more of the components of a mixture are two special features of the double-beam spectrophotometer.

Results of representative analyses were described.

Direct-Reading Analysis of Stainless and Tool Steels Covering Wide Ranges of Composition. M. F. HASLER, Applied Research Laboratories, Glendale, Calif.

The original work completed in early 1949 concerning the precise analysis of 18-8 stainless steels by direct-reading spectrochemical methods has been extended to cover a wide range of such steels especially as to determination of chromium and nickel content. Similar methods have been applied to the analysis of a variety of tool steels with marked success.

The various factors which have made possible the attainment of a new and unusual degree of accuracy for this method of analysis were discussed in detail.

Direct-Reading Methods for Analysis of Aluminum and Aluminum Alloys Using Pin Samples. R. W. CALLON AND L. P. CHARETTE, Aluminium Laboratories Limited, Arvida, Quebec, Canada.

The development of methods using an ARL Research Model Quantometer and high precision source for the analysis of aluminum and aluminum alloys was described. Reasons for choosing pin instead of disk samples were discussed. As a rapid changeover from pure metal to alloy analysis is necessary, similar source and exposure conditions are used for both types of analysis. The conditions chosen were based on the results of precision tests on samples containing Si (4 to 14%), Mg (3 to 10%), Cu (3 to 8%), Ni (1 to 3%), Fe (0.5 to 3%), and Mn (1 to 2%) as satisfactory precision can be obtained for the lower percentage constituents with a wide range of conditions. Factors which affect precision were discussed and limits of detection using optimum spectrum lines, as well as for the lines chosen, were given. The errors which result from factors which affect the accuracy of routine work but are not included in precision tests were discussed. As the instrument must be capable of analyzing 1300 samples in an 8-hour shift, minor modifications have been made. These include the use of an oscillating, water-cooled, two-position electrode stand.

Effect of Heat Treatment and Composition on Spectrographic Determination of Carbon in Steel. GEORGE WIENER, Westinghouse Research Laboratories, East Pittsburgh, Pa.

A method has been developed using commercially available spectrographic equipment for the quantitative determination of carbon in steel. A study has been made of the effects of various heat treatments and microstructures on the analytical results. Various applications to metallurgical problems were discussed. The concentration range studied is from 0.20 to 1.00% carbon in both plain carbon and low alloy steels.

Spectrochemical Determination of Magnesium in Cast Iron. GALEN PORTER, Union Carbide and Carbon Research Laboratories, Inc., Niagara Falls, N. Y.

A spectrochemical method for the determination of magnesium in the range 0.01 to 0.20% in cast iron was described. The erratic results obtained with solid samples led to the development of a solution technique which has given very satisfactory results and permitted the use of synthetic standards.

A rotating electrode assembly permits solutions to be handled as rapidly as solid samples.

Factors Influencing the Spectrographic Analysis of Silicon in Gray Iron. W. B. MCCREARY, International Harvester Co., Louisville Works, Louisville, Ky.

Louisville Works of International Harvester Company has developed a method for the spectrographic determination of silicon in gray iron, based on the use of duplicate determinations on each sample. Conditions were given under which seven samples may be analyzed for copper, manganese, and silicon in 45 minutes. Also included were eight factors which failed to produce needed improvements, and twelve factors which have contributed to the successful use of the method. Several practical hints were included.

Design and Interpretation of Two-Factor Experiments. GRANT T. WERNIMONT, Eastman Kodak Co., Kodak Park Works, Rochester, N. Y.

Chemists often make use of statistical methods to help study the results of experimental work. This leads them to see the importance of properly designing their experimental work so that clear-cut inferences can be logically drawn from the experimental results.

The following attributes are to be found in a well-designed experiment:

1. The experiment is a "sample" from some realistic population.
2. The experiment is unbiased.
3. All possible results from the experiment can be interpreted.
4. Results from the experiment can be logically combined with those from other experiments.
5. All experimental results are used efficiently.

The so-called factorial design of experiment is particularly useful because it can be made to have most, if not all, of these attributes. This paper explained the meaning which can be put into well-designed factorial experiments in which two factors are studied together. The design and interpretation of more complicated experiments follow easily for anyone who understands simple two-factor experiments.

The first example of a two-factor experiment was concerned with a simple weighing experiment in which four objects are weighed on three different scales. A comparison can be made between the three scales and the four weights. In addition, it is possible to see whether the three scales behave exactly alike when used to weigh all four objects. Failure of the scales in this respect leads to what the statisticians call an interaction or discrepance. Such interactions are often the source of trouble in a complicated test method.

A second example showed how interaction or discrepance can be used to measure experimental error. The variations in coating a thin layer of metal continuously on a sheet of steel can be studied by means of a two-factor experimental design. The systematic variations along and across the roll are compared with the interaction or discrepance which cannot be explained by either of these effects.

Two examples were used to show how real interactions between the two main factors can be detected. In a simple melting point procedure, no such interactions were found present. In a more complicated photographic experiment, interactions between the factors were found, so that the keeping properties of four kinds of film were not the same with respect to each other when processed in four different types of developer. This makes the exact interpretation of the experimental results more difficult.

The final example concerned an experiment on the lot-to-lot variation of the carbonate content of caustic soda. A two-factor experiment makes it possible to separate and compare the variability of the test method, the variability of samples taken from one position within a lot, the variation within lots, and the variation between lots. It was suggested that chemists now pay too much attention to the precision of their test methods and too little attention to the variability of sampling the materials they test.

Anomalies in Extinction Coefficients at Low Absorbancies. LIONEL S. GOLDRING, Massachusetts Institute of Technology, ROLAND C. HAWES AND ARNOLD O. BECKMAN, National Technical Laboratories, South Pasadena, Calif., CHARLES D. CORYELL, Massachusetts Institute of Technology, Cambridge, Mass., AND MICHAEL E. STICKNEY, National Technical Laboratories, South Pasadena, Calif.

Anomalies in extinction coefficients up to 7% for potassium chromate at an absorbancy of 0.1 and a wave length of 273 mμ have been reported by Vandenbelt. Anomalies at low absorbancies are always positive in sign and occur in all types of photometric instruments, according to Edisbury. Techniques contributing to most reliable measurements at low absorbancies were described. There was a discussion of possible theoretical causes for residual anomalies.

A Modified Beckman Spectrophotometer and Its Performance. D. L. TIMMA, Mound Laboratory, Miamisburg, Ohio.

A Beckman D.U. spectrophotometer has been modified by replacing the phototube receivers with a photomultiplier tube. The total instrument response, spectral purity, and spectral band pass obtainable with this instrument were compared with a standard Beckman spectrophotometer and with a Cary recording spectrophotometer. The modified instrument shows an improvement over the standard instrument and is comparable in operation to the Cary spectrophotometer.

Detection and Determination of Thianaphthene in Naphthalene. J. J. MCGOVERN AND J. R. ANDERSON, Mellon Institute, Pittsburgh, Pa.

Although naphthalene absorbs some ultraviolet radiation at 297 mμ, an examination of the spectra of thianaphthene samples prepared synthetically by different procedures and also that of a specimen isolated from crude naphthalene revealed that this compound has an intense characteristic band at this wave length. Furthermore, it has been found by the use of a suitable reference wave length and careful measurements that low concentrations of thianaphthene can be quantitatively determined in naphthalene by spectrophotometric analysis at this wave length. The concentration range investigated was that of usual interest, 0 to 4% thianaphthene. Higher concentrations should be even more readily analyzed by the same procedure. The ultraviolet spectra, analytical procedure, and calibration curve were discussed as well as sample results and comparison with chemical analysis.

Residual Monomer in Polystyrene. A Spectrophotometric Method. J. E. NEWELL, United States Rubber Co., Passaic, N. J.

A rapid method for the determination of monomeric styrene in polystyrene was described. Styrene absorbs ultraviolet radiation of 250 to 260 mμ wave length 40 to 100 times as intensely as polystyrene. By ultraviolet spectrophotometry, small amounts of monomer in polystyrene can be determined with a precision of 0.02% and an estimated accuracy of 0.05%. The method is most accurate in the absence of impurities other than styrene monomer. The presence of significant amounts of interfering substances is made evident by poor agreement of calculations for monomer from absorption measurements at several wave lengths.

Ultraviolet Absorption Analysis for *N,N'*-Di-*sec*-butyl-*p*-phenylenediamine in Gasolines. ALVIN S. GLESSNER, JR., A. C. WHITAKER, AND NORMAN D. COGGESHALL, Gulf Research and Development Co., Pittsburgh, Pa.

The oxidation inhibitor *N,N'*-di-*sec*-butyl-*p*-phenylenediamine is sometimes used in gasolines and is commonly referred to as U.O.P. Inhibitor No. 5. A method has been developed for the analysis of gasolines for this material in concentrations of the order of 1 part in 50,000. This material possesses a strong absorption band centering at about 332 mμ which was used for the analysis. A series of investigations demonstrated that although some chemical changes occur in the inhibitor as a function of time of storage, the intensity of this band is essentially constant. These were discussed. Because very low concentrations of inhibitor are usually used, it is necessary to correct for the background absorption in this spectral region due to the aromatic content. This is readily done if the inhibitor-free gasoline is available, which is generally not the case. In the latter instance it is necessary to free the gasoline of inhibitor for background determinations. This was accomplished by a technique of vigorous agitation of the sample in the presence of neutral or acidified water. The processes believed to operate in freeing the gasoline of inhibitor are conversion of inhibitor into water-soluble oxidation products which subsequently enter the water phase. The method has been in use for several years with good results on a variety of gasolines.

Determination of Anthracene in Crude Anthracene Cakes by Ultraviolet Spectrophotometry. FRANK P. HAZLETT, ROY B. HANNAN, JR., AND JOSEPH H. WELLS, Mellon Institute, Pittsburgh, Pa.

The anthracene content of a crude anthracene cake can be determined very rapidly by ultraviolet spectrophotometry. The results of some analyses made with a Cary recording spectrophotometer were compared with those of a chemical method. The discrepancies between the results from these two methods can be explained and partially eliminated.

Standardization of Spectrographic Analytical Methods. BOURDON F. SCRIBNER, National Bureau of Standards, Washington, D. C.

The desirability of making available spectrographic methods of recognized reliability has enlisted the interest of several spectrographic groups. The program of A.S.T.M. Committee E-2 in compiling suggested methods, as the first step toward standardization, has provided some practical experience in this direction. A basic principle in writing methods is to provide clear, relevant, and concise descriptions sufficiently detailed to permit reproducing the methods in other laboratories. The characteristic factors in a spectrographic method were discussed with respect to their importance and means of measurement. The essential difference between methods lies in excitation with its attendant variables of sample size, shape, and chemical or physical condition. Particular care is necessary in including pertinent details affecting excitation, as was shown by typical examples. In addition to specifying the apparatus and operating conditions for setting up a method, information on the resultant spectra obtained by the method is useful in matching the procedures in different laboratories. The following concepts, which were defined and discussed, are helpful in this respect: excitation index, exposure index, and concentration index. The standardization of certain basic features of methods, such as photography and photometry, appears promising as a beginning in general standardization of spectrographic methods.

Stability of Spectra as Determinable by Statistical Analysis of Experimental Data. J. SHERMAN, Philadelphia Naval Shipyard, Naval Base Station, Philadelphia, Pa.

Statistical analyses of the data of extended experimental investigations permit certain conclusions to be drawn concerning the stability of spectra, particularly with regard to analytical precision and its improvement. This paper discussed the nature, extent, and limitations of such conclusions, when the investigations are deliberately planned, from a statistical view. The discussion was based on results of extended laboratory investigations.

Spectrographic Determination of Beryllium in Air Dust Samples. GEORGE PETERSON, GEORGE WELFORD, AND JOHN HARLEY, U. S. Atomic Energy Commission, New York Operations Office, Health and Safety Division, New York, N. Y.

A spectrographic method for the determination of beryllium in air dust samples collected on filter papers has been developed. The procedure is applicable to all types of filters, including pleated gas mask filters which contain about 150 mg. of ash. The range covered is from 0.0025 to 10 micrograms of beryllium on the electrode, and aliquots of one twentieth of the total sample were used.

The filter paper is wet or dry ashed, treated with hydrofluoric and sulfuric acids, and taken to dryness. After solution in hydrochloric acid, the iron, aluminum, and other metals are removed by precipitation with oxine in acetate buffered solution. The excess oxine is removed with chloroform, and the beryllium precipitated as hydroxide, using 2.5 mg. of aluminum as a carrier. The hydroxide is dissolved in sulfuric acid, and the volume brought to 1 ml. of 1 to 3 acid. Aliquots of this solution are used for the spectrographic analysis.

A 0.05-ml. portion is transferred to each of three cupped, waterproofed carbon electrodes containing about 45 mg. of sodium chloride as spectrographic buffer and carrier. Excitation is with the d.c. arc at 10 amperes for 2 minutes, and the spectra are recorded on Eastman 33 plates with a Baird 3-meter spectrograph.

The average of triplicate density ratios of Be 2348/Al 2367 or Be 2650/Al 2367 are used as the analytical curve function. Check analyses show a relative error of +20%, except at the extreme lower range.

The advantages of the method over those previously published are: a more uniform matrix regardless of type of filter, the large useful range, use of triplicates with possibility of diluting remainder of solution if necessary, freedom from background on plate, and satisfactory sensitivity, even with a slow spectrograph.

Quantitative Spectrochemical Analysis of Ashes, Deposits, Liquids, and Other Miscellaneous Samples. E. K. JAYCOX, Bell Telephone Laboratories, Inc., Murray Hill, N. J.

A general technique was described which is applicable to the quantitative spectrochemical analysis of a wide variety of materials. Sample preparation, the incorporation of spectrochemical buffers, and excitation procedures were discussed for typical cases which illustrate the scope and possibilities of the method. Examples include the analysis of the ashes of rubber, plastics, paper, and cloth; deposits on walls of vacuum tubes and other surfaces; water, oils, and other liquids; and other miscellaneous solid materials.

Crucible Fusion Technique of Spectrographic Semiquantitative Analysis. J. T. Rozsa, N. A. Grondin, and J. Golland, National Spectrographic Laboratories, Cleveland, Ohio.

The feasibility of the spectrograph for semiquantitative analysis has been severely hampered by the wide variety of physical forms and molecular combinations involved. The crucible fusion technique permits a general application to refractory powders, metals, chemical salts, and solutions. The unknown is fused with an alkali or an acid flux; graphite electrodes are coated with the fused product by a dipping process. An air-interrupted spark unit, supplemented for low percentage ranges by an a.c. arc, permits detection of the 30 common elements described, and can be extended to many more.

Improved Cutting Tool for Spectroscopic Electrodes. Alfred T. Myers, U. S. Geological Survey, Washington, D. C.

The tool originally described by Myers and Brunstetter has been improved by modifying its design. In the new form it is still a combination tool built to drill, face, and turn the electrode in one operation. The new tool is a hollow brass mill frame containing two cutter bits for turning the outside cavity wall and one counterbore cutter to face the top of the crater wall as well as to hold (in the so-called pilot hole) different size drill bits for drilling the electrode cavities. The counterbore cutter is an inexpensive standard part readily obtained from commercial sources and its use in the tool allows many different shapes and diameter sizes of drill bits to be interchanged between two or more of these tools, thus allowing a great variety of craters to be cut. In spite of the number of tools now available for cutting carbon electrodes, the one described here offers simplicity of design, ease of operation, ease of adjustment, flexibility, and economy of construction and maintenance. It affords a further means of standardizing quantitative routines in d.c. arc analysis.

Spectrochemical Analysis of Bronzes by the Porous Electrode Technique. Bourdon F. Scribner and John C. Ballinger, National Bureau of Standards, Washington, D. C.

Variations in results encountered with direct spark excitation of statuary bronze samples (3 Sn, 3 Zn, 2 Pb, 2 Ni) led to the application of a solution method based on that of Feldman. The method employs low-energy spark excitation between graphite electrodes, one of which is hollow and supplies solution to the spark through a thin, porous base. Particular attention was given to rapid sample preparation and to attaining optimum conditions for high precision. A 1-gram sample of bronze drillings is dissolved by addition of 4 ml. of hydrochloric acid and 16 ml. of nitric acid and the solution is diluted to 40 ml. with water for addition to the electrode. The analysis is made with reference to standard solutions prepared either by synthesis with pure metals or by dissolving chemically analyzed bronzes. Studies of the effect of electrode shape led to the adoption of a porous electrode having a truncated-cone base which resulted in a gain of precision by a factor of 1.3 to 2.0 over the flat electrode. The standard deviations of results on the same solution, involving 100 runs averaged 20 at a time, are as follows: Pb 2.8%, Sn 3.9%, Ni 1.9%, and Zn 1.8%, for nominal concentrations of those elements. The porous electrode method offers promise as a general method for the analysis of alloys when samples and standards are not available in comparable solid forms.

Standardization in Analytical Chemistry. W. A. Kirklin, Hercules Experiment Station, Hercules Powder Co., Wilmington, Del.

Perhaps the most basic reason for the high standard of living in America today is her ability to produce high quality products in quantity. The key to this ability is standardization.

The scope and significance of standardization activities and especially the place of the analytical chemist in these activities were discussed, together with the importance of basic standards, the numerous factors involved in the standardization of analytical methods, and the interlaboratory use of these methods. New reactions, new techniques, and new instruments are the essence of progress for the analytical chemist, and he must of necessity be flexible and remain receptive to new ideas. At the same time, he must be equally aware of the importance of standardization; he must keep in mind that today's results must be comparable not only with yesterday's and tomorrow's, but also with similar results obtained in other laboratories.

Advantages of participating in the work of societies engaged in standardization, correlation of this work, and the relation of the American Chemical Society thereto were reviewed.

By focusing attention on the importance of standardization in analytical chemistry, it is hoped to create a wider appreciation of the factors involved and to develop a greater awareness of these factors by the research workers and by the instrument makers who are devising new tools for analytical work.

Separation and Analysis of Phenols by Countercurrent Distribution. Calvin Golumbic, U. S. Bureau of Mines, Pittsburgh, Pa.

When the partition coefficients of closely related compounds are appreciably different in a given system of immiscible solvents, selective extraction may be used to separate mixtures of such compounds. The Craig countercurrent distribution technique is particularly advantageous for this purpose because it employs the efficient countercurrent principle and affords a means of calculating from the distribution curves, the concentrations of individual components in a mixture. To test the applicability of the Craig method to the problem of separating and analyzing the complex phenolic fraction of coal hydrogenation oil, the partition properties of simple alkyl substituted phenols were studied. Cresols, xylenols, and ethylphenols were distributed in solvent pairs containing cyclohexane or benzene as the organic phase and 0.5 *M* alkaline phosphate buffers as the immiscible aqueous phase. At comparable pH, significant relative differences in partition coefficients were observed for each group of isomers. By countercurrent distribution in the Craig 54-tube distribution instrument, partial or complete separations among members of each group were effected.

Similarly, countercurrent distributions were made on selected high-boiling distillate fractions of the phenolic mixture obtained in the hydrogenation of Pittsburgh-bed (Bruceton) coal. Each fraction was found to contain several compounds, among which 4- and 5-indanols and their homologs usually predominated. By mathematical analysis of the distribution curves, the concentrations of these substances and of additional components were accurately determined.

Of considerable aid in the identification of the constituents of the complex phenolic fractions was the observation that isomeric phenols separate during countercurrent distribution in order of decreasing acid strength. Further study of this effect disclosed that a relationship exists between partition coefficients and ionization constants of phenols, and between these constants and the structural configuration of phenol homologs.

Low Pressure Techniques. Determination of Oxygen and Carbon in Metals. W. G. Guldner and L. A. Wooton, Bell Telephone Laboratories, Inc., Murray Hill, N. J.

Low pressure techniques have found unique applications in analytical chemistry. Among the most generally useful methods based on these techniques are the determination of carbon in metals by low pressure combustion and the determination of oxygen in metals by vacuum fusion. The application of these methods to refractory metals of very low carbon and oxygen contents has made necessary some improvements in apparatus and technique. These improvements are aimed at higher sensitivity, better control of the blank value, and the attainment of higher temperatures in an all-glass high vacuum system.

Determination of Oxygen in Zirconium Metal. E. B. Read and L. P. Zopatti, Massachusetts Institute of Technology, Cambridge, Mass.

A method for the determination of oxygen in zirconium by vaporization of the metal in hydrogen chloride gas was described. The accuracy of the procedure is determined by analysis of zirconium metal to which known amounts of oxygen were added. The presence of carbides, nitrides, and free carbon does not interfere.

Manual Polarograph for Rapid Routine Determinations. Lead and Cadmium in Zinc. L. C. Copeland and F. S. Griffith, The New Jersey Zinc Co. of Pa., Palmerton, Pa.

An inexpensive manual polarograph was described with which it is possible to determine lead and cadmium in zinc very rapidly by obtaining the diffusion currents at only three potentials. Inexperienced personnel can easily handle four samples an hour, including solution of the metal with an accuracy comparable to the more conventional spectroscopic and chemical methods. Other applications of this machine and method were mentioned.

Analytical Polarographic Study of the Bis(dialkylthiocarbamyl) Disulfides and Dialkyldithiocarbamates. EARL C. GREGG AND WILLARD P. TYLER, The B. F. Goodrich Research Center, Brecksville, Ohio.

Polarographic reduction of bis(dialkylthiocarbamyl) disulfides (the tetraalkylthiuram disulfides) and polarographic oxidation of dialkyldithiocarbamates both exhibit two waves. One of the waves in a polarogram of either type of compound has been shown to be associated with adsorption of the disulfide form at the mercury-solution interface as demonstrated by correlation between the adsorption wave and the effect of disulfide on the electrocapillary curve.

Analytical use of the polarograms requires the inclusion of the adsorption wave in any diffusion current measurements. Good diffusion current constants are obtained and either type of compound may be determined separately or estimated in mixtures, providing the pH is controlled so that neither species will be decomposed by the action of acid or base. Acid will decompose the dithiocarbamates and alkali the disulfides.

A study of the half-wave potentials of the disulfides of the ethyl and methyl homologs as a function of pH allows the estimation of K_a for the unstable free dialkyldithiocarbamic acids and also gives the equation for the half-wave potentials as a function of pH, K_a, and concentration. Mathematical development and experimental data were given.

The standard potentials, E^0, of these reversible redox systems have been determined for the ethyl and methyl homologs.

Some Uses of Tracer Techniques in Analytical Chemistry. ALLEN M. GOLDSTEIN, Tracerlab, Inc., Boston, Mass.

Radioactive tracer techniques involve the addition of radioisotopes which follow the element under consideration, either by combining with it or by being acted on by the same chemical reagent. Counting then replaces weighing or titrating. The limitations involved may include the availability and cost of the required activity, the specific activity available, and the type of radiation emitted by the useful isotope. Further limitations may be imposed by the background counting rate and, if high level activity is required, by the necessity of shielding. Inherent limitations of instruments and equipment used must also be considered.

The sensitivity of many tests is greatly increased by radiochemical methods—for example, the sensitivity of the test for bismuth by chemical means is 10^{-10} gram whereas by radiochemical methods about 10^{-15} gram may be detected. Similarly, for molybdenum, chemical methods will detect 10^{-10} gram and radiochemical methods 10^{-14} gram. However, contrary to popular opinion, chemical tests for some elements are far more sensitive than radiochemical methods. Chemical methods for iron can be made sensitive to 10^{-11} gram, and for aluminum to 10^{-10} gram. Radiochemical methods for both these elements are less sensitive by orders of magnitude.

Some analytical problems encountered in the research and development of methods for plating, extraction, elution, and distillation as well as in water and product control which may be successfully attacked by radiochemical methods were described.

Recent Applications of X-Ray Diffraction in the Analytical Field. HAROLD P. KLUG, Mellon Institute, Pittsburgh, Pa.

For more than a decade x-ray diffraction has found increasing application as a nondestructive method for the qualitative identification of substances and the quantitative estimation of solid phases. Moreover, it is frequently the only method available for certain unusual analytical problems. New applications and procedures in identification techniques involve extension to organic compounds, surface-active agents, clay analysis, and microanalysis. Most important, however, are applications to the quantitative determination of mixed phases having identical (or nearly identical) compositions. Along this line the technique is being used for estimation of the amounts of crystalline and amorphous material in stretched rubber and polymer samples, and for retained austenite in steel.

Among the advances pertaining to the precision of analytical diffraction studies are an analysis of absorption effects in quantitative determinations, an investigation of the minimum amount of crystalline substances sufficient for x-ray determination in mixtures with amorphous materials, and a precision quantitative study of binary and ternary mixtures of the anhydrous calcium carbonates.

Fluorescence Spectroscopy. WILLIAM PRIESTLEY, JR., Process Division, Esso Laboratories, Linden, N. J.

A pen recording fluorescence spectrophotometer was described using a Beckman quartz spectrometer as the monochromator, a 1P28 photomultiplier tube for high sensitivity, and a Brown Electronik pen recording potentiometer to record the fluorescence spectra. The fluorescence spectra of twelve polycyclic aromatic hydrocarbons were shown. These spectra were determined for the solid state and in benzene solution. Two types of liquid sample cells were investigated; a 1-mm. bore quartz capillary and a 1-cm. quartz absorption cell. The capillary cell was found to give higher resolution of the low wave-length peaks; however, the fluorescence intensity decayed rapidly in several cases. The large cell gave more stable and reproducible results. Resolution of the low wave-length bands was found to be greatly affected by the sample cell position.

A quantitative relationship between fluorescence intensity and concentration was found to be, $I = AC^M$ where I = intensity, C = concentration, and A and M are constants. This relationship was found to hold for most compounds in concentrations less than 0.05 gram per liter. Theoretical calculations of the spectra of binary mixtures were shown to agree with actual data.

Flame Photometer Techniques for Determining Typical Additives in Petroleum Oils. ANNE L. CONRAD AND WINIFRED C. JOHNSON, Physical and Chemical Research Division, The Standard Oil Co. (of Ohio), Cleveland, Ohio.

Flame photometer methods for analyzing lubricating oils containing additives have been developed which permit rapid and reproducible answers to be obtained with minimum effort.

The atomization of samples in organic media eliminated ashing techniques, thus decreasing possible contamination. Working in lower concentration ranges permitted samples to be diluted beyond the point where viscosity effects were evident.

Typical oils containing alkali and alkaline earth additives were analyzed, showing reproducibility. Techniques for improving accuracy have been described.

Microwave Spectroscopy and Chemical Analysis. RICHARD H. HUGHES, Westinghouse Research Laboratories, East Pittsburgh, Pa.

Although the microwave spectrograph has been used very little for analytical chemistry, its use in certain specialized applications would appear to have advantages. The general properties of molecular absorption spectra in the microwave region were described and typical spectra of linear, symmetric top, and asymmetric top molecules were shown. Brief mention was made of nuclear quadrupole effects and their importance to physical chemistry. A description of past and present analytical applications as well as future possible applications was presented. Some discussion was given of the various types of microwave spectrographs and their operation.

Tetraethyllead in Gasoline

A SYMPOSIUM on Recent Developments in Instrumental Methods for the Determination of Tetraethyllead in Gasoline was held in Washington, D. C., February 21, 1950, under the auspices of Research Division III on Elemental Analysis of Committee D-2 on Petroleum Products and Lubricants, American Society for Testing Materials. The symposium included the papers abstracted here.

Rapid Determination of Tetraethyllead in Aviation Gasoline. V. A. SMITH, W. E. DELANEY, W. J. TANCIG, AND J. C. BAILIE, Standard Oil Co. (Indiana).

The reaction of alcoholic silver nitrate with tetraethyllead to form a metallic silver colloidal suspension permits tetraethyllead determinations of polysulfide-free fuels within A.S.T.M. tolerances in 10 minutes. A photoelectric colorimeter determines the silver by turbidimetric procedures. The method has been successfully applied to aviation fuels and motor fuel stocks sweetened with copper chloride. Polysulfides interfere in doctor-sweetened stocks.

Rapid Polarographic Determination of Tetraethyllead in Gasoline. Kent A. Hansen, Thomas D. Parks, and Louis Lykken, Shell Development Co.

A polarograph is used for the direct determination of the lead ions resulting from dissolving a gasoline sample and decomposing the tetraethyllead in anhydrous Cellosolve containing hydrogen chloride. The method is generally accurate to within ±3% in the range of 0.5 to 8 ml. of tetraethyllead per gallon and requires about 30 minutes for a single determination but only 1 hour for five determinations. Peroxides or unsaturates may cause errors.

Direct-Reading Polarograph for Determination of Tetraethyllead in Gasoline. E. B. Offutt and L. V. Sorg, Standard Oil Co. (Indiana).

A simple polarograph was described for directly reading a value of from 0 to 8 ml. of tetraethyllead per gallon of gasoline on the acid extraction of the lead from a sample. Antimony is used as a pilot ion. Results are accurate to within ±0.02 ml. of tetraethyllead per gallon in the range of 0.5 to 4.0 ml. of tetraethyllead per gallon. Using four extraction vessels, sixteen determinations may be made in 8 hours.

X-Ray Methods in Analysis and Preparation of Leaded Gasoline. H. A. Liebhafsky and E. H. Winslow, General Electric Co.

This paper presented a review of the fundamental principles of x-ray absorption measurements, their promising applications, and a description of the General Electric x-ray photometer. This instrument is suitable for both analytical applications and the control of tetraethyllead blending operations.

Determination of Tetraethyllead in Gasoline by X-Ray Absorptiometry. G. Calingaert, F. W. Lamb, H. L. Miller, and G. E. Noakes, Ethyl Corp.

This paper described the refinements necessary for precisely controlling the primary voltage of the G.E. x-ray photometer in determining tetraethyllead by the absorbence of x-ray radiation. Sensitivity to voltage fluctuation is decreased by using a polystyrene block in the reference beam. The method is accurate to ±0.01 ml. of tetraethyllead per gallon when the base stock is available, otherwise accurate to ±0.05 ml. of tetraethyllead per gallon. Chief obstacle is the varying percentage of sulfur in gasoline. Methods for eliminating the problem of sulfur and halogen concentrations were discussed.

Monochromatic X-Ray Methods for Determination of Tetraethyllead in Gasoline. Harold K. Hughes and Frank P. Hochgesang, Socony-Vacuum Laboratories.

Following a description of continuous-radiation and two-line methods and the effect of sulfur in the base stock upon them, details were presented of the single-line method now in routine use. Samples are run in less than 7 minutes apiece with an accuracy which averages better than 0.1 ml. of tetraethyllead per gallon. With known base stocks, the error is 0.03 ml. of tetraethyllead per gallon. Comparisons were made of chemical, polarographic, and x-ray methods with respect to speed, accuracy, and cost.

X-Ray Fluorescence Analysis of Ethyl Fluid in Gasoline. L. S. Birks, E. J. Brooks, H. Friedman, and R. M. Roe, U. S. Naval Research Laboratory.

Analysis by x-ray fluorescence is independent of base stock or the composition of the Ethyl fluid. Primary x-ray radiation excites fluorescent x-rays in the liquid sample. This secondary radiation is analyzed by a single-crystal spectrometer and the peak intensities of proper wave lengths for lead and bromine are measured by a Geiger counter. It is expected that 10 to 20 determinations of both lead and bromine can be performed per hour.

Committee on Annual Symposia

The Committee on Annual Symposia is to be set up on a permanent basis. It will consist of six members who serve 3-year terms beginning January 1 and staggered so that the terms of two members expire each year. The editor of Analytical Chemistry and the chairman of the Division of Analytical Chemistry, American Chemical Society, will each appoint one member to the committee each year. The committee will select its own chairman for the following year, preferably about the time of the fall meeting of the American Chemical Society.

P. J. Elving has been appointed chairman for 1950 of the following committee:

	Term Expires
B. L. Clarke L. T. Hallett	1951
J. W. Stillman Edward Wichers	1952
I. M. Kolthoff P. J. Elving	1953

THE ANALYST'S CALENDAR . . .

Analytical Summer Symposium to Discuss Separations

The keynote talk at the third annual Summer Analytical Symposium, sponsored by Analytical Chemistry and the Division of Analytical Chemistry, will be given by M. G. Mellon of Purdue University, whose subject will be "The Role of Separations in Analytical Chemistry." Dates of the meeting to be held at the Chemistry Building, The Ohio State University, Columbus, Ohio, are June 16 and 17.

Ralph M. Evans, superintendent of the Color Control Department, Eastman Kodak Company, will be the dinner speaker. His topic will be "Seeing Light and Color." Charles W. Foulk, professor emeritus, Department of Chemistry, The Ohio State University, will be the toastmaster.

The program as announced by H. A. Laitinen of the University of Illinois, general chairman of the symposium, is as follows:

Friday, June 16

11:00 A.M. **The Role of Separations in Analytical Chemistry.** M. G. Mellon

12:00 M. Luncheon (advance reservation required)

1:45 P.M. **Partition Chromatography and Countercurrent Distribution.** Lyman C. Craig

2:45 **Some Theoretical Aspects of Adsorption Chromatography.** Arthur Le Rosen

3:30 Recess

3:45 **Ion Exchange Separations.** Edward R. Tompkins

4:45 **Analytical Applications of Ion Exchange Separations.** J. Schubert

6:30 Dinner Meeting. Speaker, Ralph M. Evans, superintendent of Color Control Department, Eastman Kodak Company. **Seeing Light and Color** (advance reservation required)

Saturday, June 17

9:00 A.M. **Theory and Practice in Analytical Distillation.** Arthur Rose

9:45 **Solid Solution Formation in Precipitation Reactions.** I. M. Kolthoff

10:30 Recess

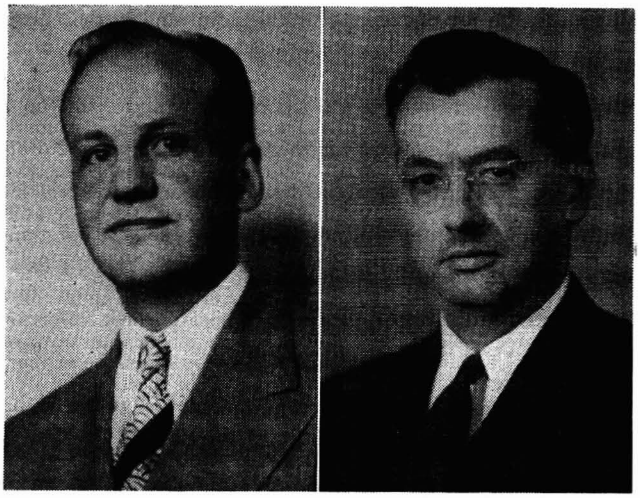
10:45 **Precipitation from Homogeneous Solution.** H. H. Willard

11:30 **Precipitation from Homogeneous Solution. Separa-**

tion of the Alkaline Earth Metals. P. J. Elving and Robert E. Van Atta
12:00 m. Luncheon (advance reservation required)
2:00 p.m. **Electrolytic Separations.** S. E. Q. Ashley
3:00 **Separation of Ultramicro Quantities of Elements by Electrodeposition.** L. B. Rogers
3:30 Recess
3:45 **The Role of Extraction in Analytical Chemistry.** George H. Morrison
4:30 **Separative Extraction of Certain Rare Earth Elements as 5,7-Dichloro-8-quinolinol Chelates.** Therald Moeller and Dale E. Jackson

REGISTRATION ARRANGEMENTS

Registration will be completed at the Chemistry Building, The Ohio State University. The registration desk will be open on Friday, June 16, from 9:00 a.m. to 5:00 p.m., and on Saturday, June 17, from 8:00 a.m. to 12:00 noon.



(*Left*) H. A. Laitinen, General Chairman of Symposium. (*Right*) W. D. Mac Nevin, Chairman of Committee on Local Arrangements

Registration fees will be $3 for individual members of the Society, designated representatives of corporation members (only one for each corporation membership), and visitors other than chemists or chemical engineers residing in the United States; $6 for nonmember chemists or chemical engineers residing in the United States, regardless of nationality. Associates of divisions or of local sections are not members of the A.C.S. and, if they are chemists or chemical engineers, are subject to the $6 fee. Full-time students of chemistry, both graduate and undergraduate, are given the courtesy of registration on the same basis as members of the Society.

HOUSING AND MEALS

It will not be possible to provide housing on the campus of Ohio State University. However, Columbus has ample hotel accommodations. The following hotels are especially recommended:

Hotel	Single	Double
Deshler-Wallick	$5.00 and up	$9.00 and up
Neil House	4.50 and up	6.50 and up
Fort Hayes	4.50 and up	7.00 and up
Seneca	3.50 and up	5.50 and up

Single rooms with twin beds are available at slightly higher prices than those quoted for doubles.

Those who expect to attend the meeting should make their reservations directly with the hotel of their choice well in advance of the meeting.

The luncheons will be held at Pomerene Hall on the campus, the price being about $1 for each luncheon. The dinner on Friday evening will be held at the Neil House at a price of about $4. Reservations for these meals must reach the local committee not later than June 10, 1950.

TRANSPORTATION

Columbus is served by five railroads, twelve intercity bus lines, and two air lines. U. S. Route 23 passes through High Street, the principal north-south street of Columbus, and U. S. Route 40 passes through Broad Street, the principal east-west street. The Ohio State University campus lies on the west side of High Street about 3 miles north of the intersection of High and Broad Streets. The Chemistry Building is located near the northeast corner of the campus between Eighteenth and Nineteenth Avenues just west of College Road. It is conveniently reached from the downtown hotel district by taking northbound High Street busses marked Arcadia, Blenheim Road, or Jeffrey Place to Woodruff Avenues, walking west one block on Woodruff Avneue to Peasley Street where a brick walk on the left leads directly to the Chemistry Building. All south-bound busses on High Street go directly to the hotel district. Pomerene Hall, where the luncheons are held, lies directly south of Mirror Lake on the campus, the entrance being on Neil Avenue.

There is only a limited amount of parking space for cars along Eighteenth and Nineteenth Avenues next to the Chemistry Building, but there is ample room in the parking lot for visitors on the east side of the Ohio Stadium within a few minutes' walk from the Chemistry Building.

American Crystallographic Association

On January 1, 1950, the activities of the American Society for X-Ray and Electron Diffraction and the Crystallographic Society of America were officially ended, and a new society, the American Crystallographic Association, came into existence. This new society will carry on the activities of both the old ones. According to its constitution, the object of this society is to promote the study of the arrangement of atoms in matter, its causes, its nature, and its consequences, and of the tools and methods used in such studies.

The charter members of the new society, totaling 496, have elected the officers for the first year, as follows: president, I. Fankuchen, Polytechnic Institute of Brooklyn; vice president, R. W. G. Wyckoff, National Institutes of Health; secretary, H. T. Evans, Jr., Philips Laboratories, Inc.; treasurer, J. Karle, Naval Research Laboratories. Meetings will be held twice yearly, and the first one is scheduled for April 10 to 12 at Pennsylvania State College, State College, Pa. Further information concerning the American Crystallographic Association may be obtained from the secretary, Howard T. Evans, Jr., Philips Laboratories, Inc., Irvington-on-Hudson, N. Y.

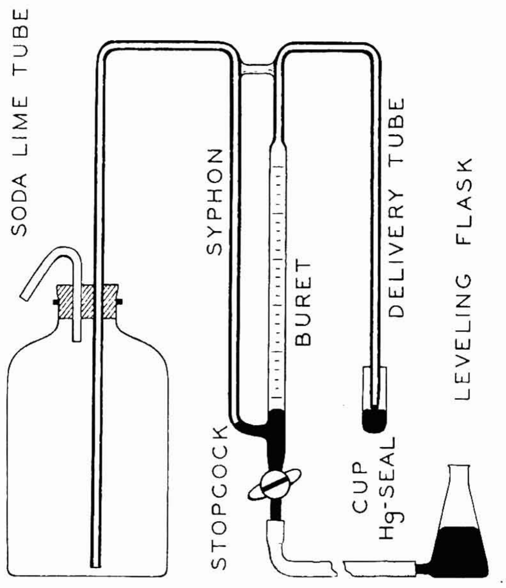
American Crystallographic Association. Pennsylvania State College, State College, Pa., April 10 to 12
Scientific Apparatus Makers of America. Chicago, Ill., May 18 to 20
Society for Applied Spectroscopy. New York, N. Y., May 26 and 27
Symposium on Molecular Structure and Spectroscopy. Mendenhall Laboratory of Physics, Ohio State University, Columbus, Ohio, June 12 to 17
Third Annual Summer Symposium. Ohio State University, Columbus, Ohio, June 16 to 17
International Microchemical Congress. Graz, Austria, July 2 to 6
Instrument Conference and Exhibit. Instrument Society of America, Buffalo, N. Y., September 18 to 22

AIDS FOR THE ANALYST . . . . .

Mercury-Sealed Buret for Dispensing Solutions from Protected Storage Bottles.

Frederic E. Holmes, Clinical Laboratory, Christ Hospital, Cincinnati, Ohio.

It may be assumed, that gases commonly present in air are insoluble in mercury or diffuse through it so slowly that the permeability of a mercury seal is in the same category with that of glass. Many types of storage vessels utilizing mercury seals for the exclusion of gases have been used to protect reagents [Holmes, F. E., Ind. Eng. Chem., Anal. Ed., 12, 425 (1940)]. In most cases the reagent, with or without measurement of volume, is delivered by displacement by mercury. In many, the flow of mercury and the volume of reagent are controlled by a stopcock located in the stream of mercury, so that the stopcock is protected from the corrosive action of alkaline solutions, deposition of solutes, and solution of the lubricant. The reagent is protected from contamination by the lubricant.



Because of the weight and cost of mercury, vessels of the older types are limited in capacity to a few hundred milliliters. Vessels of approximately 500-ml. capacity have been used, in which the reagent is displaced by measured amounts of water (unpublished), separated from the solution by a glass bell riding in a mercury seal. Care is required to avoid sticking of the bell or leakage of water past the seal when working limits of difference of pressure are exceeded.

Some modification of the physiologists' spirometer, with glass parts, may provide a mercury seal for large volumes of reagent with a relatively small volume of mercury. For many purposes a large bottle equipped with conventional protective devices is adequate. When the reagent is to be protected from oxygen or other gases not readily removed by absorption, a large light rubber bag containing nitrogen or some other inert gas, is connected with the vent of the storage bottle. The absorbent train, or bag, should produce the least possible difference in pressure in order to avoid interference with operation of the buret.

The buret shown in the diagram was mounted on a storage bottle holding 4 liters of reagent solution but with modification in dimensions, it can be used with reservoirs of much greater capacity. Advantages of smaller storage vessels have been retained: The stopcock controlling flow is in contact with mercury only; the delivery tube is protected by a mercury seal; and the desired volume of reagent is expelled by an equal volume of mercury measured on the graduations of the buret. Delivery of 10 *N* sodium hydroxide has been found sufficiently accurate for preparation of 1 *N*, 0.1 *N*, 0.01 *N*, and other alkaline solutions suitable for most purposes in the clinical laboratory.

The diagram shows the position of mercury during storage. The difference between the level in the buret and in the siphon indicates a back pressure of mercury sufficient to balance the net pressure of the solution toward the delivery tip. The potential additional pressure of mercury at this point and in the cup in which the tip is immersed, prevents accidental escape of solution.

To obtain a measured volume of reagent the cup is removed, the leveling flask is hung in a position slightly above the top of the buret, and mercury is admitted until it reaches the zero mark. In this operation, the mercury in the tip, and usually 1 or 2 drops of solution, are expelled. With a suitable receiver in place, mercury is again admitted until the desired amount of reagent has been delivered. The final volume is approached at a moderately slow rate to prevent oscillation of mercury between the buret and the siphon, especially if the reagent is being delivered below the surface of other liquids in the receiver.

To refill the buret, the cup is placed over the tip, the leveling flask is lowered to the table, and the stopcock is opened. Mercury is drawn up in the delivery tube and falls in the siphon until reagent enters the buret. The reagent then flows past the mercury while the latter drains from the buret into the flask. The stopcock is closed when the mercury reaches a point about 0.4 ml. below the zero mark. During refilling, the mercury in the delivery tube falls back to the cup.

The buret is a 25-ml. graduated pipet of borosilicate glass. Siphon and delivery tubes are of capillary tubing, approximately 2 and 1.5 mm., respectively. The over-all height is approximately 700 mm. The tip of the delivery tube is about 175 mm. and the junction of siphon and buret is about 150 mm. above the table top. The distance between the junction of the siphon with the buret and the lowest graduation is 20 to 25 mm.

Stainless Steel Boats for Carbon Determination.

Ralph Carlson, American Cast Iron Pipe Company, Birmingham 2, Ala.

Because of the short life of clay combustion boats, cast stainless steel combustion boats were made and used in order to lower laboratory costs. The author's laboratory runs total carbon on iron and steel by the direct combustion method, using 1-gram samples with ferric oxide as a bedding material and granular tin as an accelerator. The temperature of the combustion furnace is 2000° to 2100° F. and time in furnace is that specified by the A.S.T.M. procedure.

The boats, which are made of Type 310 stainless steel (25% chromium–20% nickel), are 3.5 inches long, $^{11}/_{16}$ inch wide, and $^{1}/_{2}$ inch high, and wall thickness is $^{1}/_{8}$ inch with ends $^{3}/_{16}$ inch thick. They are easily cast in snap molds.

During the 6 months this type of boat has been used, the average life has been about 500 determinations per boat. At an estimated cost of about 50 cents each, the boat cost per determination was about 0.1 cent. Clay boats averaged eight determinations per boat, and cost 31 cents or 3.87 cents a determination. Use of the steel boat saves several hundred dollars a year.

The stainless steel boats carry no more blank than the clay boats, and the oxide bed and fused sample do not adhere to them.

The operating temperature and subsequent air cool with each determination produce ideal annealing conditions for this type of metal, which doubtless add to the life of the boats.