ANALYTICAL CHEMISTRY

Volume 22, No. 1 Copyright 1950 by American Chemical Society Issued January 18, 1950

SECOND ANNUAL REVIEW OF ANALYTICAL CHEMISTRY
FUNDAMENTAL ANALYSIS

END OF REVIEW SECTION

MICROCHEMISTRY

NOTES ON ANALYTICAL PROCEDURES

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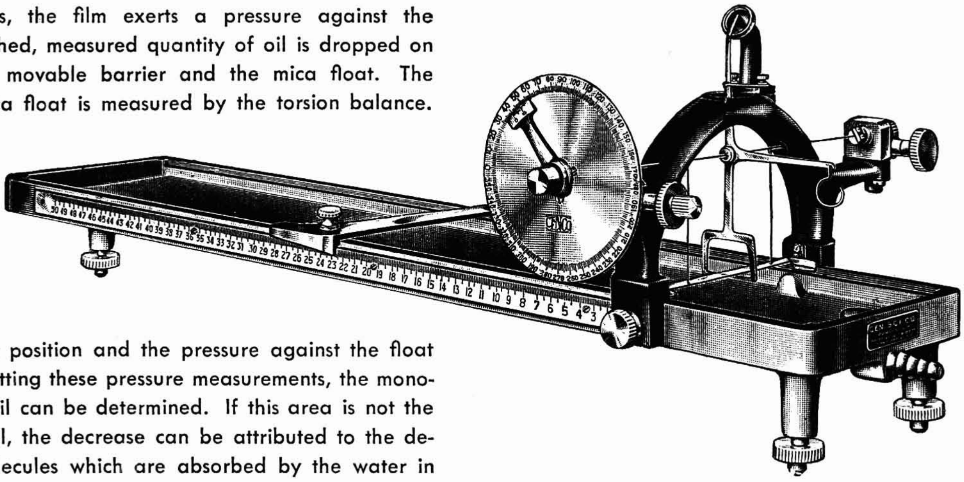

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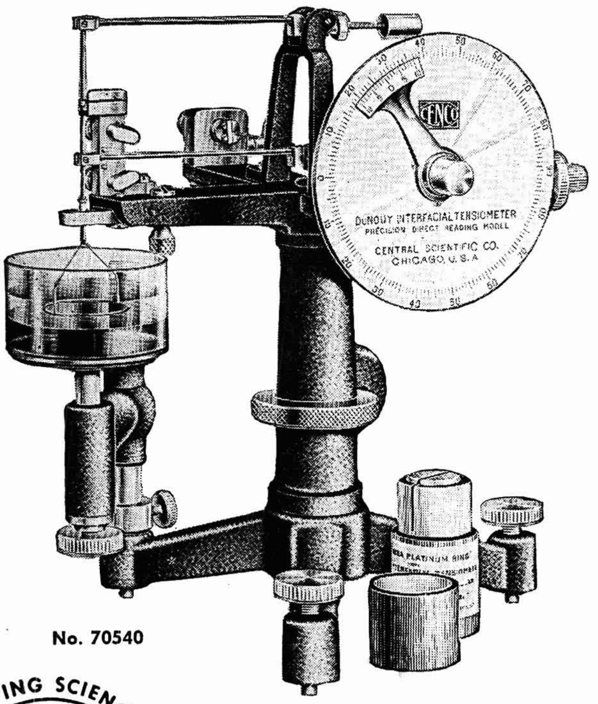

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Issued February 15, 1950

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FUNDAMENTAL ANALYSIS

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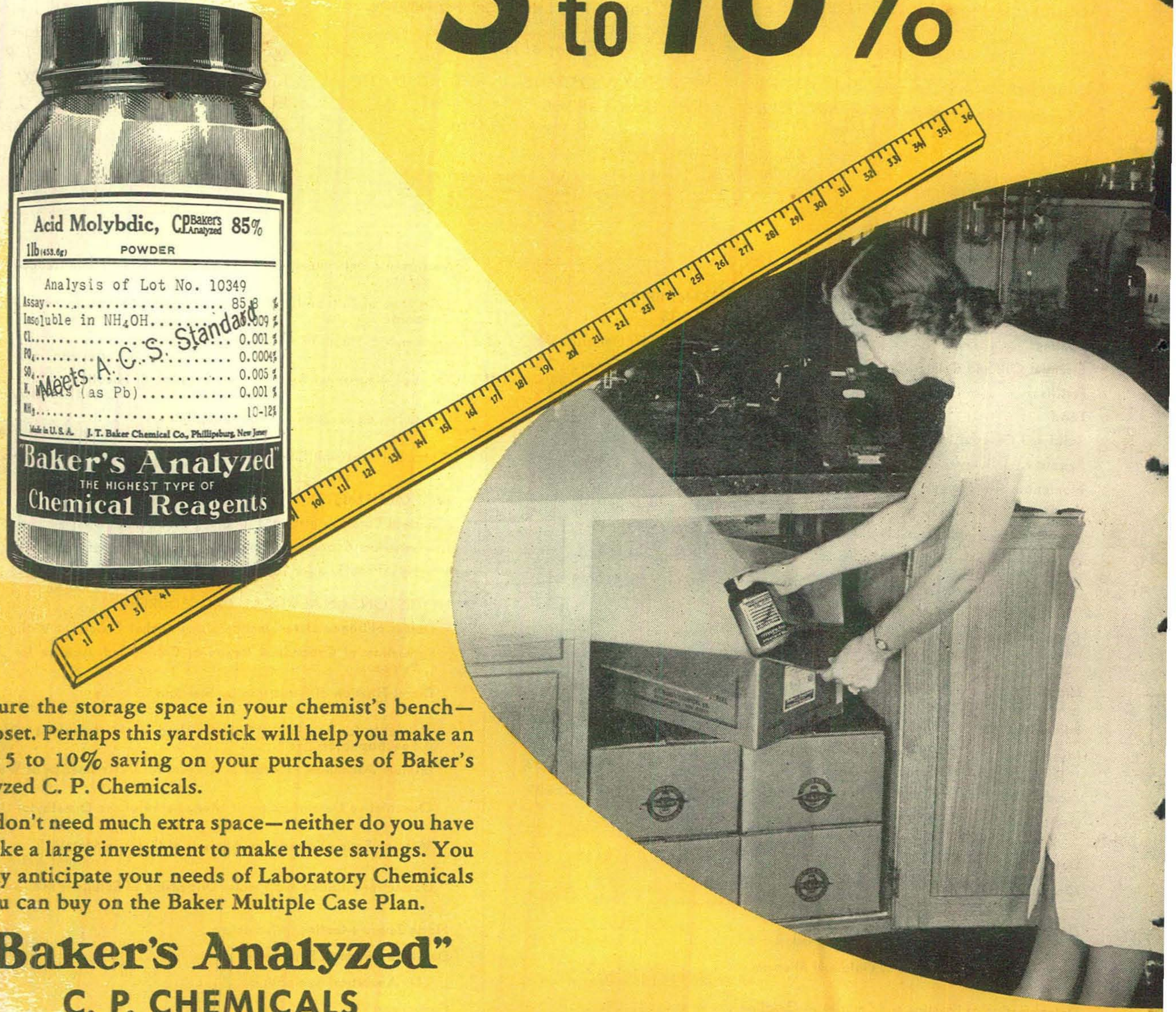

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END OF SYMPOSIUM

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Round-Table Discussions:

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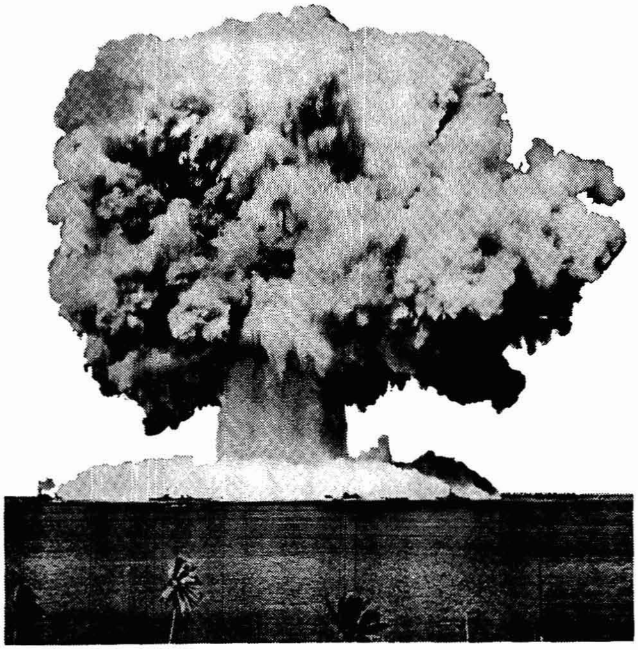

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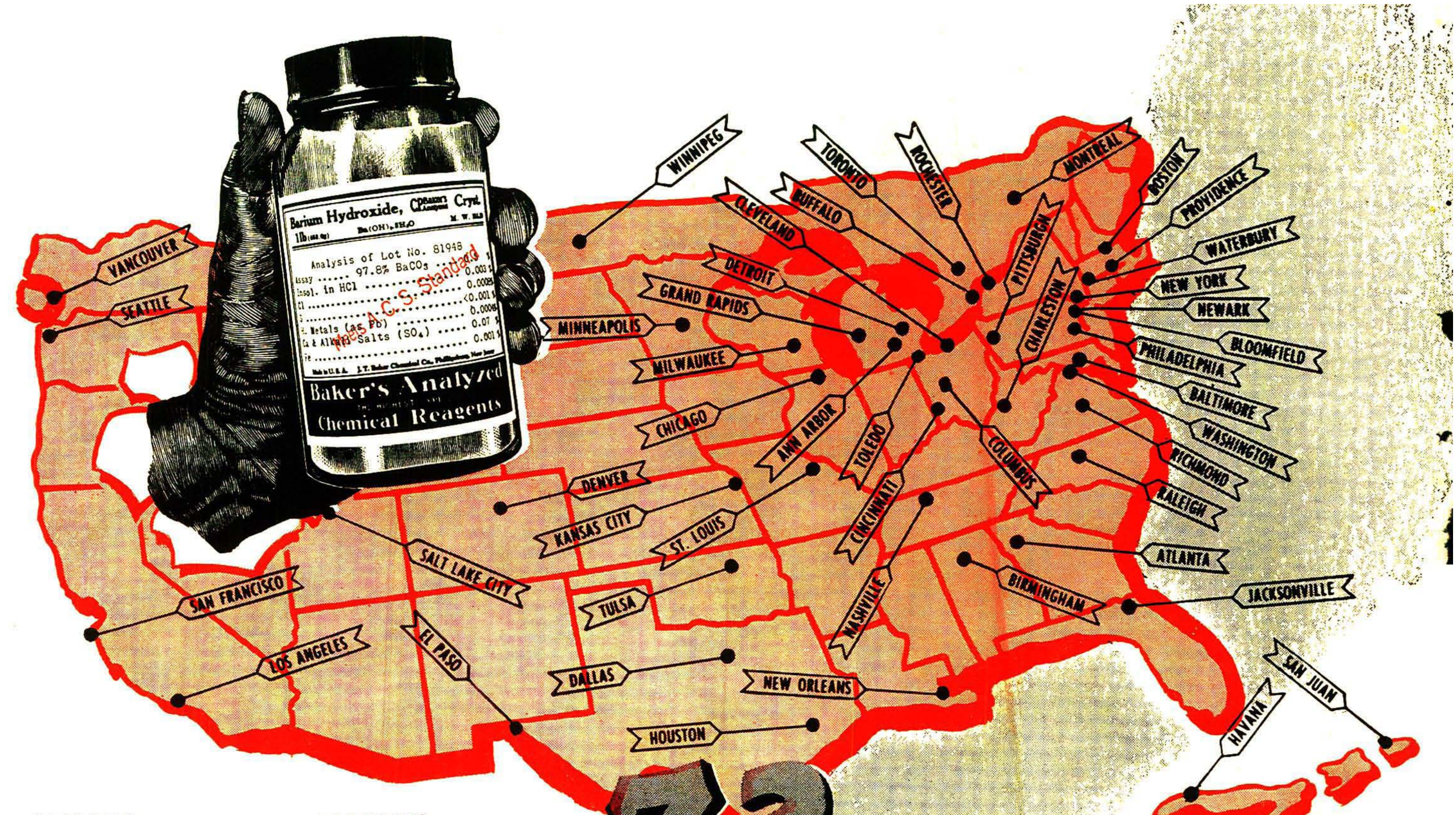

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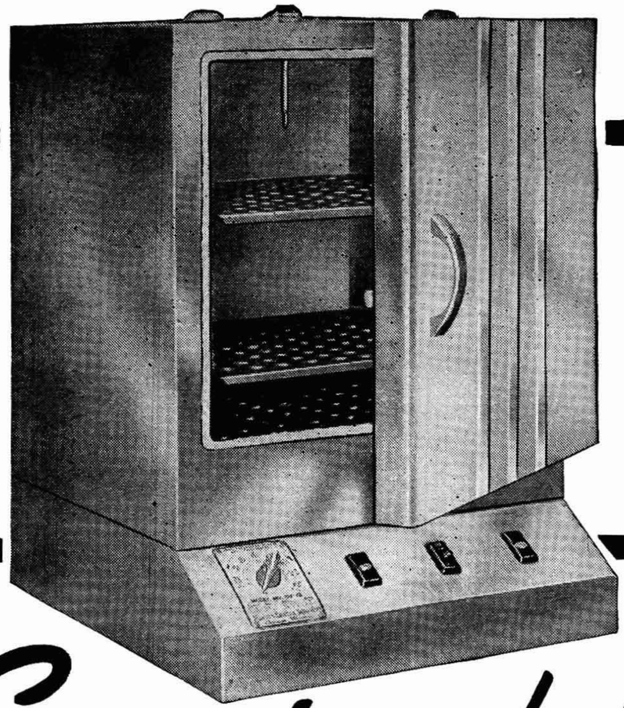

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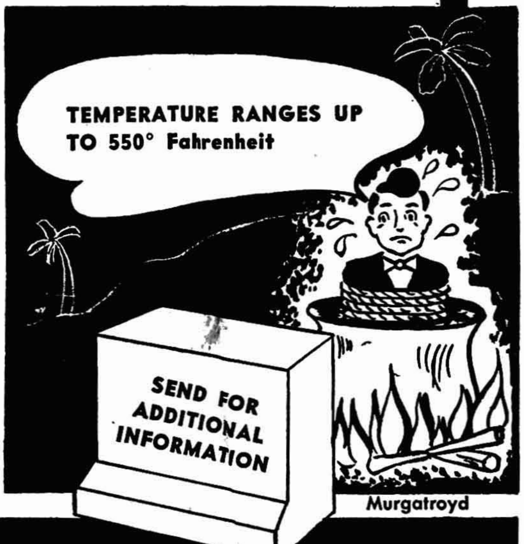

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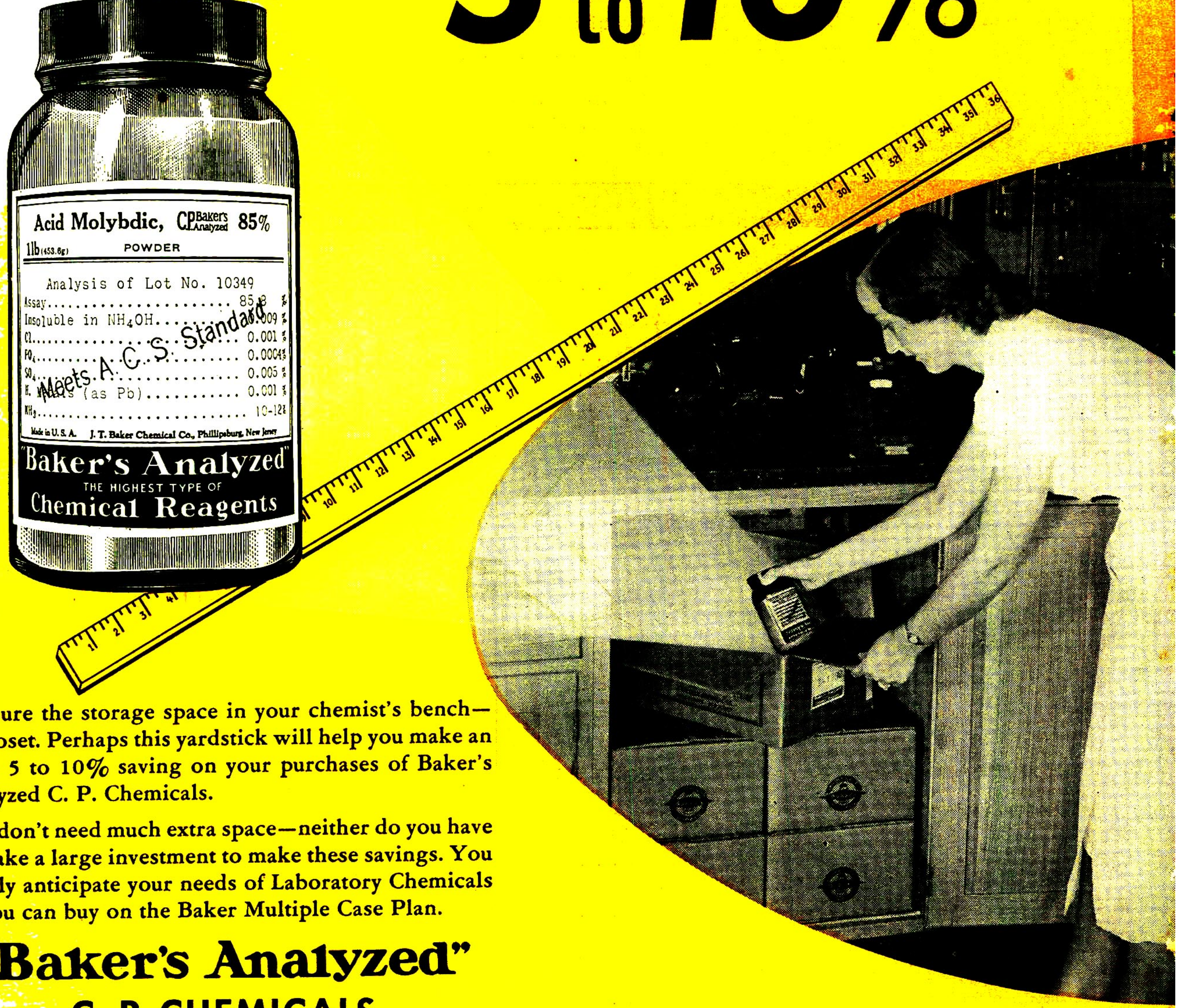

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Adsorption Symposium

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MICROCHEMISTRY

NOTES ON ANALYTICAL PROCEDURES

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MICROCHEMISTRY

ROUND-TABLE DISCUSSION

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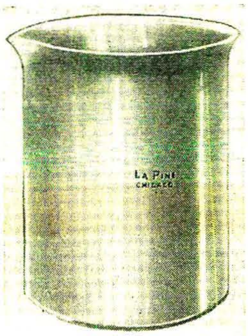

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3000 ml	$5\frac{7}{8}$	$6\frac{7}{8}$	**3.55**	**3.20**
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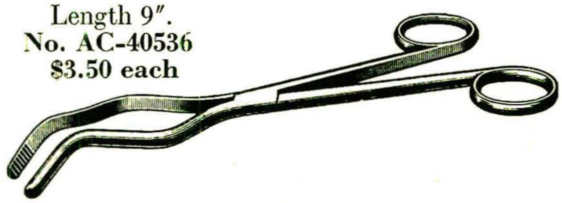

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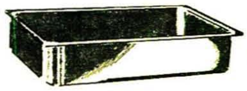

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SYMPOSIUM ON TETRAETHYLLEAD IN GASOLINE

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SEPARATIONS IN ANALYTICAL CHEMISTRY. Third Annual Summer Symposium

MICROCHEMISTRY

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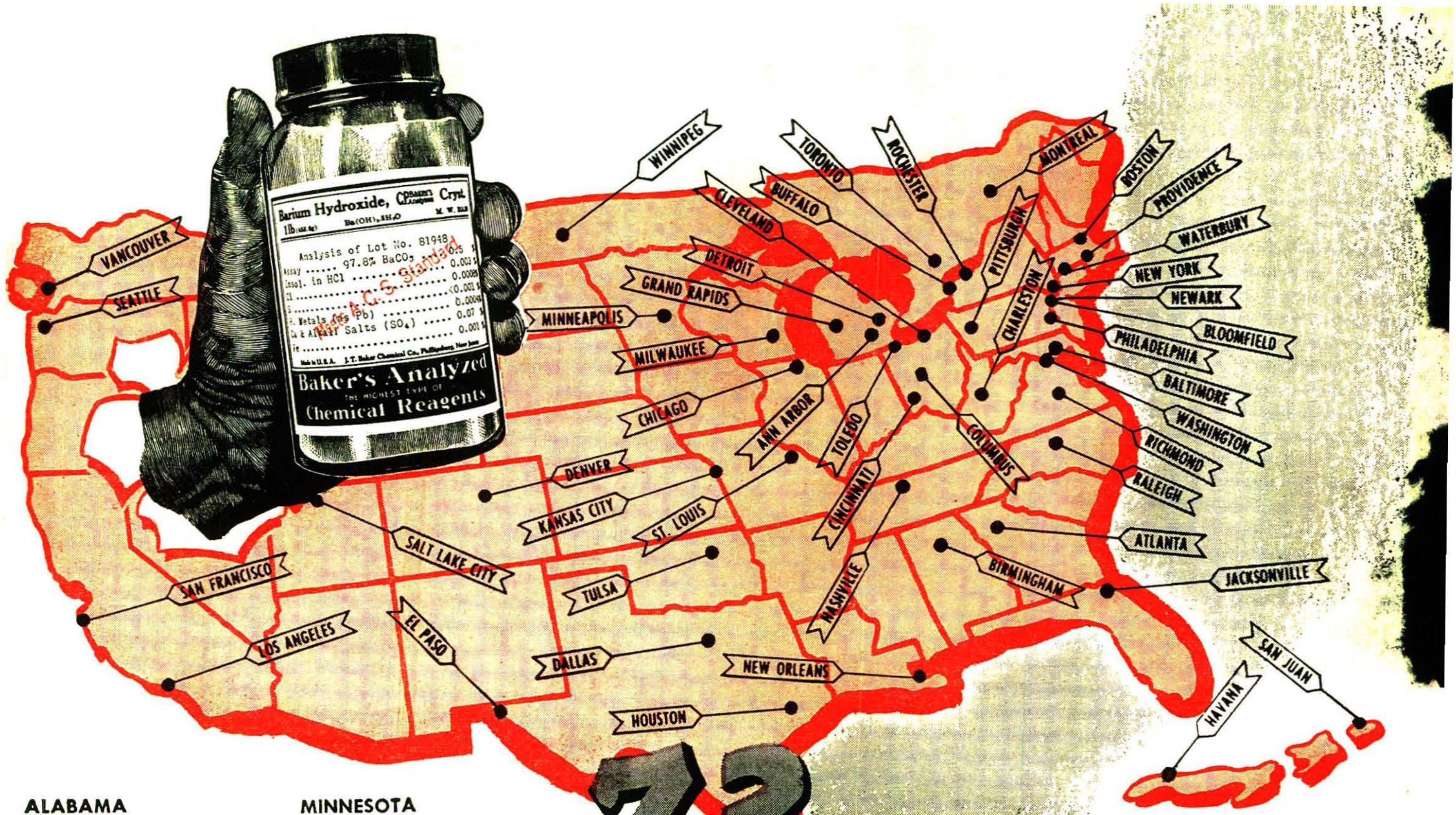

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ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

An Expanding Program of Special Symposia

Analytical chemists will not lack opportunities during the first half of 1950 to exchange scientific and technical information. The Third Symposium on Analytical Chemistry of Louisiana State University, to be held January 30 to February 2 at Baton Rouge, La., the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, to be staged in the William Penn Hotel, February 15 to 17, the Fifth Annual Microchemical Symposium, to be conducted at the American Museum of Natural History in New York on February 24 and 25, and the Third Annual Summer Symposium, cosponsored by the A.C.S. Division of Analytical Chemistry and Analytical Chemistry, are likely to attract record attendances.

Our neighbor to the north, the Chemical Institute of Canada, will stage its second analytical symposium in the Royal Connaught Hotel, Hamilton, Ontario, on March 2 and 3. Presentation of the Fisher Award to I. M. Kolthoff, and a symposium on chemical analysis by means of infrared spectrophotometry, will feature a four-day program of the Division of Analytical Chemistry at Houston, the last week in March.

The renaissance in analytical chemistry is worldwide. Word has reached us that the Austrian Society for Microchemistry has definitely settled on July 2 to 6, 1950, as the date for the First International Microchemical Congress at Graz, and plans are maturing satisfactorily for an International Congress on Analytical Chemistry, to be held in Great Britain sometime in 1952, under the patronage of the International Union of Pure and Applied Chemistry.

What a contrast these activities provide when comparison is made with the situation prevailing but a few years ago, when analytical chemistry was looked down upon by most of the chemical profession and avoided as one would a plague. The continued successes of special symposia demonstrate conclusively that analytical chemistry is no longer destined for a Cinderella role.

Analysts are to be congratulated for the initiative and leadership required to operate so many and varied symposia. Industry is sympathetic to these gatherings, for it senses that more accurate, more rapid, more precise methods of analysis will make possible introduction of new large scale continuous processes, better products, and much-needed economies in manufacturing. It realizes also that new and revolutionary methods of analysis can develop from but one source—increased research of a fundamental nature.

The Challenge of Tomorrow

The Pittsburgh Section of the American Chemical Society, in bestowing the Pittsburgh Award on H. V. Churchill, has honored all analytical chemists, for he typifies all the finest qualities and attributes of the profession.

Over the years he has made many notable scientific contributions to the field of analytical chemistry, but over and above strictly scientific considerations, he has provided at all times constructive leadership and sound judgment in professional problems of direct concern to analysts. His geniality, his kindly humor, his profound understanding of human nature, are widely known throughout the entire chemical profession. The mere listing of Mr. Churchill as toastmaster is positive assurance that a dinner or meeting will be a success. In his humble way he has taught analysts that there is nothing unprofessional about a good hearty laugh.

The philosophy of Mr. Churchill is easily discernible in the following quotation from his speech of acceptance.

> At meetings such as this, there is a strong temptation to be reminiscent and to recall the days of the past. Upon the occasion of an award such as this of tonight, a review of the past is probably necessary to justify or explain the action of the award jury, but in the field of science, whether it be pure or applied, it is always morning—the beginning of tomorrow. It is a time when "dawn stands tiptoe on the misty mountain-top." Out there lies a field of active work, achievement, and accomplishment. In my happy experience the tasks that lie ahead—immediate and future—have always been inviting. While there is much that has been done, there is more to do. The challenge of the future is very real to all men whose life work is along scientific and technological lines. I yield to no one in my respect and admiration of the great, the near great, and the unnamed workers who laid the foundation and built the structure that has brought analytical chemistry to its present high estate—and yet, analytical chemistry, today, is but a promise of what is to come.

The part H. V. Churchill has played in the great resurgence that has brought analytical chemistry to its present position of high dignity, esteem, and recognized importance in the hierarchy of both pure and applied chemistry is gratefully acknowledged by the members of the profession.

2nd Annual

Review of Analytical Chemistry

ONE year ago we inaugurated this series of review articles to make widely available the latest developments in the field of analysis. In that first group of articles the authors covered a five-year period. The theoretical or fundamental material was given in 29 articles in the January 1949 issue and the practical applications of new developments in various fields of specialization were reviewed in 11 articles in the February issue.

The same general plan has been followed in the preparation of this second review except that only a one-year period, 1949, is covered. The fundamental developments are given in 27 articles on the following pages of this issue, and the practical applications will be written up in 11 articles in the February issue.

Because we believe that these reviews contain valuable material for the analyst in his day to day work, a combined reprint of the 1949 articles comprising 260 pages was made up for ready reference. Copies of this reprint are still available at $1.50 per copy from the reprint department, AMERICAN CHEMICAL SOCIETY. Reprints of the reviews in the January and February 1950 issues will also be available at a later date. Advance orders at a price of $1.50 will be accepted.

Once again we wish to call attention to the generous contributions of time and effort by the authors in making these reviews possible. Our sincere thanks and appreciation go to each and every one of them.

—The Editors

LIGHT ABSORPTION SPECTROMETRY

M. G. MELLON, *Purdue University, Lafayette, Ind.*

THE outline followed here is substantially that used earlier (*94*). This means that the material included is considered as being primarily chemistry or physics. Under chemistry come the items dealing with whatever transformations are necessary, in the form of preparative treatment, to get the sample material in condition for measurement. Under physics come the items dealing with the operation of measurement, which means instruments and their use.

The chemistry and physics involved concern methods as methods. Thus, the old thiocyanate method for iron may be studied from the viewpoint of determining the variable factors influencing the development of the color and/or the instrumental arrangements and operations best suited for measuring the colored system. Another kind of study concerns the application of such a method, under specified experimental conditions, to the determination of a constituent in some specific kind of material, as, for example, the determination of iron in a beryllium bronze by the thiocyanate method. In the latter case usually the major problem is the chemical control of interfering reactions, although it might be the measurement of a multicomponent colored system. In general, the reviewer acquiesces in the recommendation of physicists to restrict the word "colorimetry" to the determination of color as color, or color specification. Qualitative and quantitative analyses based on the determination of the light absorptivity of systems then belong under light absorptiometry. Absorptiometry is listed as a dynamic property method in a new classification of analytical methods (*105*).

There seems to be no decrease in the annual volume of publications dealing in some way with light absorption spectrometry. Because space cannot be taken to appraise the contribution of each of the hundreds of separate items, this review is limited to mention of what seem the most significant kinds of publications. No doubt in many cases the references selected as representative of the various kinds of contributions could have been replaced by others equally as, or more, satisfactory.

CHEMISTRY

As in all other subdivisions of quantitative analysis, the work in this subdivision neither knows nor uses any particular kind o chemistry. Fundamentally there is only one kind—that of th chemical elements of the periodic table and of combinations o two or more of these elements in the form of radicals or compounds. The analyst simply selects the reagents and the reactions which can be turned to use in ascertaining the qualitative and/or quantitative composition of a given material.

Work in this category seems to fall into two fairly well separated groups: study of various means of preparation of colored systems for measurement, including occasionally systems for permanent standards; and study of the nature of the color-forming process, including the effect and possible control of any variable factors affecting the final color desired.

Preparation of Systems for Measurement. From the standpoint of simplicity and efficiency, the analyst prefers a self-colored desired constituent. If this system is colorimetrically satisfactory and if there is no interference, direct measurement is possible. Such cases, which involve no preparative chemistry, occur infrequently.

Whenever this ideal situation is not found, at least one preparative operation is necessary. Colorless constituents must be rendered measurable in the visual region of the spectrum through some appropriate chemical reaction which gives a colored system or changes one of known value already formed. Interfering constituents are rendered noninterfering through complexation, separation, or compensation. With increasing recognition, then, of the possibilities of absorptiometric measurements, the search goes on for systems more nearly approaching the ideal for such work (*95*).

Adaptation of Known Reactions and Reagents. In general, the kind of item included here is a reaction already known. Its use may become feasible through the discovery of some means of providing for interference. New possibilities of control of some variable factor affecting the reaction may provide for situations otherwise troublesome. The adaptation may be chiefly a refinement in technique in handling various operations. The basic contribution of many publications is of this nature.

Thus, Cooper and Winter (*27*) have improved the method for the determination of vanadium as vanadotungstophosphate. Keenan and Flick (*73*) have adapted the reaction of nitroso R salt with cobalt to the determination of the metal in atmospheric dust. Silverman (*118*) has recommended specific operative details for using dithizone as a reagent for lead in high purity copper. A "reversion" process for using this reagent for certain metals has been employed by Irving *et al.* (*67*). The precipitation separation of aluminum as oxinate has been utilized by Wiberley and Bassett (*128*) for the colorimetric determination of the metal, following extraction of the precipitate with chloroform.

The spectrophotometric determination of hydrogen sulfide (*43*) is based upon the known action of this compound to give an equivalent amount of methylene blue under certain conditions. The observation by Swank (*121*) that aluminum interferes in the determination of iron with ferron is the basis of the recommendations to complex both metals and then to measure the aluminum at 370 mμ and the iron at 600 mμ (*28*). Somewhat similar is the use of 1,2-dihydroxybenzene-3,5-disulfonate for iron and titanium (*107*). Following measurement at 560 mμ, the iron complex is bleached with sodium dithionate and the titanium measured at 399 mμ.

The reactions of hydrogen peroxide to give percolumbic acid and of thiocyanate to give a tungsten complex have been adapted (*51*) to the determination of these two metals in aliquots of a solution containing both elements.

The rarely used colloidal dispersion is the basis of a method for determining silver (*63*). The procedure is based on the old observation that sugar reduces a dilute solution of silver ions.

New Reactions and Reagents. The search for new reagents or reactions is motivated by a need either for one not previously available, or for one which in some respect will be an improvement over any already known. In the case of organic reagents often the new one is simply a derivative of an old one, such as a substituted 1,10-phenanthroline. The factual knowledge accumulated in this way is impressive, but the really important contributions are disappointingly low. Perhaps more physical chemistry is needed to enable one to predict effects beforehand—for example, the effect of a given substituent, such as fluorine on the chelating capacity of a compound, such as 1,10-phenanthroline. Now we laboriously make the reagent and then test its action.

In a new indirect method (*89*) sulfate is precipitated as $Co(NH_3)_6BrSO_4$ by means of hexamminocobaltic bromide. Colorimetric measurement of the cobalt in an aqueous solution of the precipitate serves for calculation of the sulfate. A different kind of indirect method serves for nitrite (*22*). The thiocyanic acid liberated by reaction of the nitrite with urea is complexed with a known excess of ferric ion to give the measurable color system.

A method for determining gold in ores and concentrates (*88*) involves various preparative steps, including separation of the gold by precipitation and extraction. Finally the gold is complexed as the tetrabromoaurate ion for measurement.

New methods for iron continue, that of Shome (*116*) being based upon complexation of the ion with isonitrosodimethyldihydroresorcinol. A number of common complexers for iron, such as fluoride, phosphate, and citrate, are stated not to interfere.

Determination of copper with dithio-oxamide (*127, 129*) involves the use both of gum arabic to stabilize the sol formed and of malonic acid to buffer the solution and to prevent certain interferences.

An example of capitalizing on an interference reaction is illustrated in the method of Davenport and Thomason (*29*) for determining uranium with thioglycolate. In his study on the use of this reagent for determining iron Swank (*122*) had reported the interference by uranium.

The difficulties encountered by the analyst working with complicated organic systems are illustrated in a method for the determination of benzylpenicillin (*18*). Involved, closely prescribed preparative and separative processes precede the reaction of the desired constituent with hydroxylamine to give a colored system for measurement.

Permanent Standards. The use of various simple kinds of materials as permanent standards for numerous colorimetric applications continues.

For pH determinations over the range of 2 to 10, mixtures of solutions of chromium, cobalt, copper, and iron are recommended, using Bogen's universal indicator (*42*). Another pH measurement is represented in neutralimetric titrimetry with methyl yellow or methyl orange as indicators (*101*). Mixtures of solutions of potassium dichromate and cobaltous sulfate are recommended for the end-point colors in daylight.

Although the work is no longer new, it may be well to remind analysts of the data obtained at the National Bureau of Standards on the transmission values for a solution of potassium chromate (*61*). The use of high purity potassium dichromate (N.B.S. standard sample 136) provides a ready means for preparing this solution to check photometric scales of instruments.

Interpretative Study of Colored Systems. Perhaps no analyst understands fully all the intricacies of even the simple systems he prepares for measurement. If the over-all procedure gives desired results, he may not actually need much understanding. But to the extent that the action of variable factors affects the reliability of the result, careful work necessitates recognition of the factors operative in a given case and of means utilizable for their control, as far as this is possible.

Interpretation of the nature of the chemical systems analysts employ is the province of physical chemistry. To the extent that this kind of information becomes available, analysts profit. Such understanding is the basis for knowing what to expect, for instance, when dealing with the heteropoly compounds, pH-sensitive color-forming reagents, or instable systems. Some examples of interpretative studies considered analytically significant are included here. As might be expected for light absorptiometric work, they concern primarily the nature of complexes and the conditions for their formation.

Korenman (*77*) has discussed the general theory of certain organic analytical reagents, and specifically organic reagents for boric acid. Kuznetsov (*79*) reviewed color formation with organic reagents on the basis of the internal ionization in molecules containing distinct dipole character. Recommendations were made for improving such reagents.

Molybdo- and tungstoheteropoly acids are considered in detail by Jean (*70*). Both molybdenum and tungsten blues, prepared from heteropoly acids containing arsenic, phosphorus, or silicon as the central atom, were studied by Vaĭsberg and Daĭn (*124*). Molybdenum blues from the same heteropoly acids gave anomalous absorbencies for Clausen and Schroyer (*25*) between 400 and 800 mμ.

The constitution of cobalt(II) halogen complexes in acetone was studied by Barvinok (*11*), and of copper(II) chloride in aqueous solution by Brown (*21*).

Light absorption measurements were used by Souchay and Peschanski (*119*) in the study of condensation phenomena involving the formation of complexes. For pH-sensitive reactions A. K. Babko (*5*) decided that the range favorable to achieving maximum color can be calculated from the instability-constant of the complex and the dissociation constant of the complex-forming acid.

Okáč and Polster, after studying the red solutions obtained on oxidation of the nickel salt of dimethylglyoxime, concluded that the oxidation occurs on the oxime group rather than on the nickel (*103*, *104*). Such chelate-forming compounds have engaged the interest of other workers. Calvin and Melchior (*23*) studied the relative stabilities of the 5-salicylaldehyde sulfonates of cobalt (II), copper(II), nickel, and zinc. Lee, Kolthoff, and Leussing (*81*) determined the kinetics of formation and dissociation of ferrous-1,10-phenanthroline. Others (*13*, *78*) have studied the conditions affecting the formation and decomposition of ferrous-2,2′-bipyridine. The chelating possibilities of certain amino acids were reported by Martell and Bersworth (*90*).

The general question of the mechanism of the reduction of complex ions, some of which are colored, was studied by Fridman (*47*).

For certain molybdate, tungstate, and vanadate ions Feigl (*38*) studied the possibilities of complexation with acidic fluoride solutions, along with decomplexation of the systems so formed by means of boric acid. In studying the effect of foreign ions on the determination of metals A. K. Babko (*6*) concluded that the ratio of the dissociation constant of the desired complex should exceed that of each of the interfering complexes by a factor of well over 1000 for satisfactory results, if the interfering complex is colored. H. Babko and Rychkova (*7*) reported specific results of this kind for salicylate complexes of aluminum.

The work of Lanford and Quinan (*80*) established the nature of the reaction of ferric iron and citric acid. Similar work of Foley and Anderson concerned complex formation of sulfosalicylic acid with uranium (*44*) and with copper (*45*).

Experiments with azure blue dye solutions led Segal (*113*) to the conclusion that absorption in the red end of the spectrum, where most deviation from Beer's law was noted, is a function of the degree of ionization of the coloring material.

PHYSICS

Because every absorptiometric method involves measurement, the importance of the physics of instruments and their application is inescapable. Fortunately, physicists and engineers have done reasonably well in providing adequate equipment. Of course, one always wants devices which are at least better, more nearly automatic, or cheaper than those now available. It seems safe to state, however, that the chemistry of getting many constituents ready to measure is far behind the physics of the instruments for the measurement.

Four new books may be mentioned which deal, at least in part, with spectrometric instruments and their applications. Lothian's book (*83*) covers various equipment employing visual, photographic, photoelectric, and radiometric receptors for transmitted or reflected radiant energy. It may be taken as representative of British viewpoint and symbolism. The work of Körtum (*76*) is a German book on general colorimetry (absorptiometry) and spectrophotometry. The American book by Harrison, Lord, and Loofbourow (*59*) contains much practical information on general spectrometry, with major emphasis on emission work. The most comprehensive book on absorptiometry, edited by the reviewer (*96*), is a composite work, of which only the chapter on photographic techniques is not written by an American. The material covers the chemistry concerned in the preparative treatment of the sample material, instruments for the spectral range from 0.2 to 25 microns, and their general applications, including color specification.

Instrumentation. Instrumental contributions deal primarily with improvements in, and adaptations of, previously described kinds of apparatus.

Preston (*108*) has reviewed progress in photoelectric cells, with special reference to their use in photometry and radiometry. Jones has suggested a new classification system for detectors of radiant energy (*71*), together with a discussion of factors of merit for such detectors (*72*). Macbeth and Nickerson (*87*) have summarized the spectral characteristics of various light sources.

Miller *et al.* (*100*) have described the new Beckman (Model B) spectrophotometer, mentioned a year ago. Recently announced accessories for the Beckman DU instrument include a test tube holder, a temperature controller, a short path flow cell, and a 50-cm. absorption cell. Recommendations from the National Bureau of Standards deal with standards for checking the calibration of spectrophotometers (*52*). The problem of correcting for slit width errors in precision work with monochromators has been considered again (*58*). An auxiliary working standard has been proposed as equivalent to magnesium oxide on a General Electric spectrophotometer (*48*).

Several new filter photometers are available. The Fisher Nefluorophotometer, a two-cell (photovoltaic) instrument designed to measure light transmitted, reflected, or emitted by a sample in solution or suspension, is stated to serve as a colorimeter, nephelometer, or fluorometer (*41*). The Ruoy photometer (*82*) is a one-cell (photovoltaic), 10-filter instrument precalibrated for many clinical test methods. By reference to a table accompanying the instructions provided for each such constituent, the amount may be read for a given scale value. The Coleman (*26*) colorimeter (Model 8) may serve somewhat the same clinical purpose with its six filters. Its ordinary absorbency-transmittancy scales are interchangeable with special scales serving for various constituents. Adaptors provide for the use of semimicro and micro absorption cells. Reinhold (*109*) has suggested a direct-reading technique for any standard scale instrument. The Farrand fluorometer (*37*) has a photomultiplier tube and uses either plain or interference glass filters. Stross (*120*) has extended the range of measurable absorbence on the Spekker absorptiometer.

Two patents are of some interest. Gumaer's instrument (*55*) serves for analyzing a fluid mixture by means of transmitted light, and Merckel's color-test device (*97*) is a simple comparator, presumably designed for field tests.

A new tristimulus integrator has been developed (*30*) for use with the General Electric recording spectrophotometer. By providing tristimulus values as the spectrophotometric curve is being drawn, accurate to ±0.001, this device makes practical the application of numerical methods to the solution of color problems. Already Godlove (*53*) has reported its use for various problems, such as statistical tests, small color difference colorimetry, and different kinds of dye phenomena. This accessory equipment is now commercially available for the instrument. Gummed spectrum strips, useful for explaining spectrophotometric curves, are now available for paper of the size used in the General Electric instrument.

An improved photometric unit, with exposure heads designed for reflectance measurements under various conditions, has been announced (*49*).

Theory of Measurement. Coordinate with consideration of instruments as such is consideration of the laws basic to the kind of measurement concerned and of items related to the most effective application of the procedure.

In a discussion of the validity of Beer's law Hardy and Young (*56*) present data for a conforming two-component dye solution, including a method for analyzing such a multicomponent system.

Körtum (*75*) has evaluated the accuracy of visual absorbency measurements made near the ends of the visible spectrum.

Instead of the familiar Beer's law calibration curves Ayres (*4*) recommends Ringbom's method in which absorbency (1 — transmittancy) is plotted against the logarithm of the concentration. Inspection of such a curve shows the optimum range for a given photometric error—that is, the concentration range having the steepest slope. Use of transmittancy values directly, which constitute the usual data on many spectrophotometers, gives curves the mirror image of the absorbency curves.

An algebraic method has been suggested (*123*) for correction for absorption by interfering constituents not readily handled chemically or instrumentally. The underlying assumption is that the absorbency of the interferer(s) can be represented by an analytical function of wave length, and that this function does not represent the absorbency of any of the components being determined.

Sacconi's method of indirect colorimetry (*112*) was devised for systems in equilibrium, comprising two substances differing in absorptivity and having an equilibrium constant, but not being readily separable.

A new offset method of interpreting spectrophotometric curves (*117*) provides for qualitative and quantitative analyses by direct visual comparison of the curves linear in logarithm of absorbency and plotted on transparent paper.

A color synthesizer provides (*125*) for visual demonstration of the color corresponding to a given spectrophotometric curve.

APPLICATIONS

Whatever there may be in the way of a new or improved technique, the final objective is the application of the procedure to a practical problem. Much of the material being published represents relatively minor contributions. Among the many papers, however, appear certain kinds of significant applications.

Chemical Analysis. The use of the thiocyanate ion for complexing the ferric ion is one of the classic color-forming reactions. Bezel (*15*) has applied it to the analysis of waters containing part of the iron complexed with humic acids. This old reagent has been applied to the determination of columbium (*2*) and of rhenium (*50, 93*).

The determination of manganese in steel as permanganate is the subject of a report by a committee of the British Iron and Steel Research Association (*98*). Measurement in this form is the basis of a method for determining manganese compounds in air (*39*). A variation in application is the proposal of Andersson (*3*) to standardize dilute (0.01 *N*) permanganate solutions by measurement of their light absorptivity.

Methods involving heteropoly compounds indicate the continuing absorptiometric versatility of these systems. Thus, phosphorus may be determined in phosphate rock as molybdovanadophosphoric acid (*10*) and in steel by reduction of molybdophosphoric acid to molybdenum blue with hydrazine sulfate (*1*) or with chlorostannous acid (*60*). Egsgaard (*34*) recommends amidol for forming the blue solution. The method of Brabson *et al.* (*19*) provides for the determination of silica by reduction of molybdosilicic acid to molybdenum blue in the presence of fluorine and phosphorus. Molybdenum blue seems to be involved in a method for columbium in steel (*31*). Molybdogermanic acid, reduced to molybdenum blue by hydroquinone, is the basis for a method for germanium in silicates (*40*). Similarly, molybdoarsenic acid reduced to molybdenum blue by hydrazine sulfate serves for copper in copper-base alloys (*24*). The use of tungstosilicic acid in a method for ascorbic acid (*8*) illustrates the application of heteropoly acids for determining certain reducing substances through the formation of heteropoly blues. Benham (*14*) determined glucose similarly, but with the use of molybdophosphoric acid. Englis and Miles (*35*) established conditions for using the mixed molybdenum-tungsten reagent for determining levulose in the presence of dextrose. Rather than reduce such a heteropoly acid to a blue, some analysts prefer to measure the free acid itself, as in the determination of silicon as molybdosilicic acid in hydrofluoric acid (*32*) or in steel (*64*).

The diversity of current absorptiometric methods is illustrated by the determination of antimony in alloys as the tetraiodoantimonate (*102*), of copper in tin- and lead-base alloys as diethyldithiocarbamate (*115*), and of copper in aluminum and zinc die-casting alloys as the basic citrate (*54*). Multicomponent colored systems may be handled absorptiometrically for chromium, manganese, molybdenum, nickel, and phosphorus in gray iron (*62*), and for chromium, molybdenum, nickel, and phosphorus in other metals (*1*). A two-color system is involved in the method for dichromate in the presence of chromate (*91*).

The many possible determinations of organic compounds, in addition to those just mentioned, are illustrated by procedures for epinephrine (*33*) using ferrous sulfate, and for certain pyrimidines (*114*) using 2-thiobarbituric acid. Conversely, the latter reagent may be determined by means of its reaction with 2-sulfaniloamidopyrimidine. Beznák (*16*) employed ratio measurements in his spectrophotometric method for the simultaneous determination of hemoglobin and myoglobin.

Occasional use is made of color-diminution methods, wherein fading or decrease of a standard color system is a known function of the amount of some constituent reacting with the desired constituent, or something equivalent to it. This is the general principle of the methods of Klement for calcium (*74*), of Rusconi *et al.* for magnesium (*111*), and of Rider for thorium (*110*).

Slowly evidence accumulates to indicate the feasibility of employing at least some spectrophotometric methods to ranges of concentrations usually thought to be the domain of common gravimetric or titrimetric procedures. With kojic acid as the complexer (*92*), Mehlig has continued his work of handling ores containing up to 58% of iron. Bastian (*12*) reported successful determination of 78% of copper in a bronze with a Beckman (DU) instrument. He set the absorbency scale for zero with a solution containing 15 grams of copper per liter, using a spectral band width of 25 mμ to increase the light intensity. The solutions of the copper samples were then read against this "blank." This kind of measurement is in essence an application of the recommendation of Hiskey (*65*) for measuring the sample against a similar system of fairly high absorbence rather than against pure solvent.

Color Specification. As stated by R. M. Evans, color (as color) "sprawls across the three enormous subjects of physics, physiology, and psychology." His superbly done book, mentioned previously, deals with color from these viewpoints. Complementing it, Bouma's new book (*17*) deals with the purely physical aspects of color.

Probably the most generally used method of specifying colors, the use of words, remains the least definite, primarily because too many people mean too many different things by the same word. In the hope of alleviating this difficulty somewhat, the Inter-Society Color Council has issued a comprehensive list of color terms (*66*).

Another very old procedure is color matching in terms of some kind of material color standards. The British Colour Council is issuing a new dictionary (samples) of colors (*20*), the specific purpose being for interior decoration. Jacobson's new book (*68*) is a detailed description of the Ostwald system of color specification. A new, enlarged edition has been issued for the Color Harmony Manual, which is based upon the Ostwald system (*69*). Still another example of a color matching set is the South American collection of 7279 samples in the atlas of Villalobos-Dominguez and Villalobos (*126*). The Plochere standards (*106*) consist of 1248 painted papers, the colors of which are produced by systematic pigment mixtures. Middleton (*99*) has reported comparison of them with chips of the well-known Munsell system. The general considerations underlying all such color-order systems have been treated by Foss (*46*).

Barnes (*9*) has discussed problems of measuring color, including the suggestion of ten specific points of application for plant problems involving color.

Lykken and Rae (*85*) have recommended the measurement of the absorbency of colored materials, such as lubricating oils, relative to the absorbency of a liquid color standard selected to approximate the hue and absorbency of the unknown. For their work the standard solutions were mixtures of iron(III) and cobalt(II) chlorides, acidified with hydrochloric acid. Comparative results were obtained with five different filter photometers. In an earlier publication Lykken (*84*) recommended glass standards for such measurements. Evans *et al.* (*36*) have considered the general methods of color measurement used in the petroleum and allied industries.

MacAdam (*86*) investigated the possibilities, in terms of distribution of chromaticities, of making a variety of colors by mixing various concentrations of suitably selected colorants. Results were computed for 171 pairs of typical dyes.

When the spectral characteristics of the standard and the sample are similar, it has been found feasible (*57*) to specify color by measurement at a single selected ordinate by means of a photoelectric colorimeter.

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INFRARED SPECTROSCOPY

ROBERT C. GORE, *American Cyanamid Company, Stamford, Conn.*

IN THE first annual review of analytical chemistry (*18*) it was reported that some 1000 papers dealing with infrared spectroscopy had appeared since 1943. During 1948 more than 300 papers with bearing on the field were published. It appears likely that this upward trend in popularity will remain with infrared spectroscopy as more and more chemists discover its utility.

If it is possible to observe developmental tendencies in the field over the passage of one year the following could be considered as important: The increasing recognition by many chemists of the infrared spectrum of a compound as an important physical property is shown by the fact that in one issue of the *Journal of the American Chemical Society* 33 spectra were reproduced. Papers on instrument design are decreasing, while those dealing with accessories and techniques involving novel application of the field are increasing. Empirical qualitative studies are being published from many new workers in the field on many different classes of compounds. Quantitative analytical papers, representing only about 7% of the total published, are not stressing general principles as often as reporting specific analyses. Fundamental vibrational analyses with attendant calculation of thermodynamic functions are on the increase, even though most of the simpler molecules have been treated.

A more detailed review of the published work during the year follows but no attempt is made to cite exhaustively all publications. Lecomte (*155*) and Sutherland (*240*) have also published reviews.

BOOKS

Early in the year "Practical Spectroscopy" by Harrison, Lord, and Loofbourow (*105*) appeared with a useful treatment of infrared spectroscopy, especially from the experimental standpoint. Bhagavantam and Venkatarayudu (*29*) published a book on the "Theory of Groups and Its Application to Physical Problems." The monograph on "The Chemistry of Penicillin" (*43*) included an extensive chapter on the infrared work done on that problem both here and in England. In France Lecomte published two volumes (*152, 156*), the first on biological applications, and the second on infrared spectrometry and its physicochemical applications. "Infrared Determination of Organic Structures" by Randall, Fowler, Fuson, and Dangl (*208*) treats the subject from the standpoint of a practicing spectroscopist and offers many suggestions for beginners. This work reproduces 355 spectra mainly obtained while the authors were engaged with the penicillin problem.

INSTRUMENTATION

During the year there were few major advances in instrumentation. The manufacturers of all commercial spectrometers began the production of percentage transmission recording instruments following the lead of Hilger and Baird. The new Perkin-Elmer Model 21 appears to be an exceptionally versatile instrument with such features as variable scanning time, automatic suppression, and relatively simple electronics. The Beckman instrument employs the unique system in which a recorded trace of the spectrum of the source is played back while the sample spectrum is being taken. A new 13-cycle alternating current amplifier, developed by Perkin-Elmer for use with modulated beam spectrometers, has advantages in stability, linearity, and freedom from beat and pickup over the breaker-type amplifier. A similar amplifier is described by Brown (*33*).

Radiation sources have shown little change, but there is renewed interest in the Nernst glower (*186*). There has been further study of the Globar (*229*). Finkelnburg (*72*) has carefully discussed the conditions for black-body radiation in gases. It has been customary to blame many of the difficulties of infrared spectroscopy on the relative inefficiency of the radiation sources and the insensitivity of the usual broad-band detectors. Golay (*87*) has described a method of multislit spectrometry which increases the over-all efficiency of a spectrometer over that obtained from the usual two-slit monochromator. The correction of slit width errors has been discussed by Hardy and Young (*104*).

Radiation detectors have come in for their share of study with important articles by Amdur (*6*), Daly and Sutherland (*54*), Golay (*88*), and Jones (*123–125*). Jones has exhaustively discussed the ultimate sensitivity, factors of merit, and a proposed system of classifying radiation detectors.

Recording systems of the conventional type are treated by several authors (*34, 64, 120*), while oscillographic recording is described by others (*36, 128, 188*). Lecomte (*153*) has revived the evaporographic technique of J. F. W. Herschel and M. Czerny to record infrared effects.

The advent of research in the low temperature field has led several investigators to describe low temperature cells (*166, 214*) of both transmission and reflection types. For use especially in biological work a microcell has been devised (*268*) as well as a soldered cell (*138*), a cell constructed of fluoropolymer (*137*), and a holder for filamentous materials (*117*). Perhaps silicon and germanium may find some uses as cell windows, for they have been shown to transmit fairly well beyond 2 microns (*20, 21, 32*).

Reflecting microscopical studies (*17, 28, 37, 66, 91, 94, 95, 148*), although not extensive in the infrared region, show promise in reducing the size of sample necessary to obtain a spectrum, as well as in biological studies and crystal structure investigations.

POLARIZATION AND CRYSTAL STUDIES

In the future, polarization data will be taken as a matter of course for the information they yield in solid phase studies, just as spectroscopists now often run a sample in several states and solvents and with several prism materials. The present novelty of polarization studies, perhaps, offers justification for the assembly of this special section at this time.

Transmission polarizers continue to be the devices of choice, with Perkin-Elmer now offering one as an accessory to its spectrometers. For those desiring to construct their own polarizers according to the method of Elliott, Ambrose, and Temple which utilizes selenium films, it has been reported that the evaporation substrate may be Formvar (*7*) rather than the intractable solutions of nitrocellulose. There are experimenters who still prefer the chemical reactivity and scattering losses found in silver chloride films to the fragility of selenium.

Because methods of orienting crystalline samples for polarization studies are often troublesome, it is worth while to attempt orientation by polishing and adsorption on suitable surfaces (*10*). Proteins (*4*), polymers (*5, 70*), and silkworm gut (*90*) have all been subjected to polarization studies with varying results. A field of great utility is in the vibrational analysis of molecules, where polarization measurements in the solid state aid materially in the assignment of frequencies (*100*).

The theories of the spectra of crystals have received further attention this year with general papers by several authors (*58, 119, 154, 214*). Walsh and Willis (*258*) have reported no change in band width on cooling several samples to low temperatures, an observation that appears to be in disagreement with those of others. Individual crystal studies include ammonium and thallous nitrate (*132*), ammonium halides (*257*), silicates (*172*), naphthalene (*206*), *n*-butyl alcohol (*13*), and β-quartz (*217*).

QUALITATIVE ANALYSIS

Spectroscopists engaged in qualitative analysis or molecular structure studies, who must search for published spectra, are confronted with a multiplicity of journals now publishing these spectra. This outgrowth of the widespread realization of the value of infrared data by chemists in many different fields will plague spectroscopists until compound indexes or extensive collections of standard spectra are available. Space limitations make it impossible to list here all spectra published during the year, even though these are of prime importance in infrared analysis. Spectra reported in other sections of this review are not listed here again, but must be sought under the different headings.

Qualitative studies were reported on the following molecules or classes of compounds: alcohols and aldehydes (*11*), amino-substituted α,β-unsaturated ketones (*52*), anthraquinone (*73*), benzene hexachloride (gamma) (*53*), benzoyl cyanide dimer (*171*), the carbonyl linkage (*107*), carboxylic acids (*209*), cyclopropane and cyclobutane (*61, 200*), ketones (*210*), methyl isolinoleate (*157*), minerals (*151*), naphthalene (*255*), organic compounds from 22 to 39 microns (*199*), 11- and 12-oxygenated steroids (*126*), penicillic acid (*187*), Pennsylvania lubricating oils (*77*), phenyl and cyclohexyl eicosanes (*191*), quinoline thiols (*103*), silicones (*213*), sulfones and related compounds (*219*), thiazine derivatives (*165*), thiophosphoryl chloride (*41*), and uracil, 5-chlorouracil, and thymine (*144*).

QUANTITATIVE ANALYSIS

Few general papers in this branch have appeared during the year. A discussion of the evaluation of accuracy in photometric analysis (*14*) belongs in this class, along with the description of a rapid method of computing spectra with a manually operated potentiometer and recorder (*75, 272*). Tyler (*254*) gives a method of computing the correct cell thickness to give optimum absorption. Starr and Lane (*237*) discuss the accuracy and precision of light hydrocarbon analyses. Studies on pressure broadening (*9, 169, 173, 174*) are of certain interest in gas analysis. Special instruments such as multipoint and gas analyzers have also been discussed (*76, 139, 180, 266*).

The following specific quantitative analyses were discussed: impurities in *n*-heptane concentrates (*8*), dimethyl ether in methyl chloride (*42*), assay of the procaine salt of benzylpenicillin (*48*), estrone, equilin, and equilenin (*40*), ester carbonyl (*102*), benzene hexachloride (*170, 252, 265*), one method utilizing a mass isotope dilution method, gaseous hydrocarbons (*181*), vitamin D_2 (*197*), and leucine isomers in protein hydrolyzates (*56*). Fry reports the use of infrared analysis in pilot plant control (*79*).

HYDROGEN BONDING

The influence of hydrogen bonding on specific absorption bands has been recorded in many articles during the year, but specific studies on bonding have been few. These include alcohol-amine association (*15*), mononitronaphthylamines (*108*), bonding in some 4-substituted cyclohexanols (*196*), the nature of the bond in potassium hydrogen fluoride (*264*), and the presence of bonding in 4-triacetyl-D-xylosidaminopyrimidines (*35*).

BIOLOGICAL

An interesting and thought-provoking study is that reported by Miles and Beck (*19, 179*) on olfaction in bees. Essentially, their hypothesis is that the smell of substances, which do not react chemically with the olfactory organs, is dependent upon their infrared absorption between 6 and 12 microns.

Most of the other papers of biological flavor might well be relegated to the other sections of this report. For the benefit of rapid scanners, however, these articles include studies on synthetic polypeptides (*12*), nucleic acids, nucleotides, and nucleosides (*30*), cancerous tissue (*31*), proteins (*55*), deuterium-labeled steroids (*63*), aqueous solutions of organic acids and salts (*92*), antibiotics derived from *Bacillus polymyxa* (*93*), native spruce lignin (*122*), the amide linkage in a native globular protein (*141*), and natural proteins (albumin) and related substances (*142*).

POLYMERS

Hampton (*101*) has described an infrared analysis for cis-trans forms in low temperature polymers; similar studies were reported by Hart (*106*) and Treumann (*253*). Studies on polymers at 4° K. are reported by King (*136*), and Kellner (*133*) has analyzed the vibrations of long-chain methylene groups in polythene. Saunders and Smith (*218*) discuss the spectra and structure of Hevea and gutta elastomers in an interesting article.

REACTIONS AND COMBUSTION

Although the techniques of reaction studies in the infrared region are not as simple as those in the ultraviolet, some studies are now beginning to appear. Rates of deuterium exchange and isomerization (*62, 92*), a comparison of carbonyl frequencies with reaction rates (*74*), the autoxidation of linseed oil (*118*), the chlorination of bicycloheptane (*215*), and the hydrolysis of starch grains by polarized radiation (*221*) were reported during the year. Infrared researches on combustion were made by several investigators (*3, 112, 230, 231*).

ASTROPHYSICAL

As is usual, there was interest shown in analyses of the atmosphere and related astrophysical subjects (*1, 2, 22, 81, 167, 177, 178*).

THEORETICAL AND MOLECULAR

The number of papers in this category is customarily the largest. Moreover, as methods of study develop and increased interest is manifested, this work must expand. Much of the work is on the chemistry-physics borderline but must be considered as the ultimate goal in many cases of analysis. Certainly, the thermodynamic studies are of major importance to the practicing chemist and chemical engineer.

These papers have been divided roughly into two classes. The first, the theoretical class, contains the more general papers while the second, the molecular class, contains papers dealing primarily with specific molecules. This, of course, does not mean that there is no overlapping of the two groups.

The theoretical papers include studies on cis-trans isomerism (*23*), identical atomic groups (*46*), homonuclear molecular absorption induced by intermolecular forces (*51, 194, 263*), forces and force constants (*130, 159, 160, 233, 247, 248, 250*), integrals useful in molecular orbital calculations (*195*), redundant coordinates (*239*), electronic states of conjugated systems (*45, 249*), interaction terms (*67, 149, 159*), acyclic molecules (*59*), degenerate fundamentals (*60*), the asymmetric rotor (*89, 98*), condensed systems (the solid state) (*267*), internal rotation (*129, 135, 150, 182, 184, 185*), the iteration method of solving secular equations (*143*), the planar XY_3 molecule (*109*), the tetrahedral anions (*110*), the octahedral XY_6 molecule (*111*), overlapping spectral lines (*190*), the Christiansen filter effect in slurries of organic crystals (*205*), intensity measurements (*212*), developments in the theory of secular equations (*246*), the classification of symmetry coordinates (*243*), symmetrical triatomic molecules (*245*), and the deformation vibrations of hydrogen atoms attached to double bonds (*227*).

The specific molecular studies include papers on the following: hydrocarbon molecules in general (*44, 80, 82–85, 228, 234*), benzene (*16, 49, 50*), methane (*38, 114, 161, 189, 238, 244, 256*), halogenated and deuteromethane, the deutero ammonias (*39*), ethane, including halogenated and deuteroethane (*24, 25, 27, 69, 183, 201, 224, 235*), propane, including deuterated (*78, 168*), *sec*-butyl alcohol (*26*), boron compounds (*47, 203, 204, 261*), ethylene, including halogenated (*68, 145, 146, 158, 251*), acetylene and methyl acetylene (*175, 176, 192, 271, 273*), allene (*113*), ketene (*65*), hydrogen halides (*57, 193, 260*), dimethyl cadmium, mercury and zinc (*96, 97*), ethylene oxide (*86, 259*), carbon monoxide (*115, 147*), carbon dioxide (*222*), carbonyl fluoride (*270*), hydrogen cyanide (*116*), malonitrile (*100*), ethyl alcohol (*99*), chlorine trifluoride (*121*), tritium deuteride (*127*), urea (*131*), the octenes (*140*), formaldehyde (*158*), thiophene (*162*), arsine and deuteroarsine (*163, 164*), acetaldehyde (*198*), oxygen fluoride (*202*), the C_3 to C_5 diolefins and styrene (*134*), cyclopentene and cyclohexene (*71*), diazomethane (*207*), nitrous oxide (*211*), the picolines (*216*), hydrazine (*220*), vinyl acetylene (*236*), the C_4 hydrocarbons (*223*), ethyl mercaptan (*224*), thioacetic acid (*226*), methyl and dimethylbutanes (*241*), ethylbenzene (*242*), the C_6 hydrocarbons (*225*), polythene (*232*), inorganic molecules and complex ions (*262*), octatetraene (*269*), and oxalyl chloride (*274*).

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RAMAN SPECTRA

W. G. BRAUN and **M. R. FENSKE**, *The Pennsylvania State College, State College, Pa.*

THE literature of the past year on analytical Raman spectroscopy has been principally devoted to the extension of the method in relatively narrow fields. Few changes in technique or improvements in instrumentation have been reported.

Brief reviews of the subject have been given by Robert (*83*) and Barceló (*2*). A chapter in the book by Harrison *et al.* (*38*) offers a good discussion of the methods used.

APPARATUS

A principal instrumental development of the year has been the introduction of a Raman spectrograph by the Lane-Wells Company. This integrated equipment (Figure 1) consists of a stabilized source and a pen recording spectrometer that can also be used as a photographic spectrograph. The packaged unit, furnished complete with all essential accessories, is said to be suitable for operation by laboratory technicians and makes available to industry Raman equipment designed primarily for analytical work.

The use of electron multiplier tubes in Raman spectroscopy has been reviewed (*45*) and a brief description of a photoelectric spectrograph used industrially has appeared (*39*).

Kirby-Smith and Jones (*46*) have described a method of preparing optically clear fluorothene scattering tubes of superior chemical resistance for handling fluorine compounds.

ANALYTICAL PROCEDURES

In general, analytical Raman spectroscopy discussed in the literature during the past year has been done using the established procedures outlined briefly in last year's review (*7*).

Heigl *et al.* (*39*) have developed an interesting method for the determination of total olefins and total aromatics in hydrocarbon mixtures. Usual analyses for individual compounds are made by determining the intensity (peak height) of selected lines. However, in the analysis of complex hydrocarbon mixtures for total aromatic or total olefin content, the Raman peaks characteristic of the olefinic and aromatic double bond are the result of a large number of individual compounds and because these lines vary slightly in position among the various compounds, a broad band is recorded at the position characteristic of each of these bond types. These authors established that the area under a recorded peak can be employed as a measurement of the scattering power, and that this is proportional to the concentration of the characteristic compound type. Using these scattering areas the minimum detectable aromatic content was found to be approximately 5 volume % and the minimum detectable olefin content corresponded to the unsaturation present in a sample containing 5 volume % hexenes.

This method should prove of great value to petroleum chemists because it provides results that may be obtained rapidly and generally independently of the other constituents of the sample. It could possibly be extended to the estimation of other groups which have characteristic Raman lines such as the $\mathrm{C{=}O}$, $\mathrm{C{-}Br}$, $\mathrm{C{-}Cl}$, $\mathrm{C{\equiv}C}$, and $\mathrm{C{\equiv}N}$ groups.

Tunnicliff *et al.* (*95*) have described an algebraic method for the correction of interfering absorption in spectrophotometric analyses, which may be of value in correcting Raman spectra for the effects of fluorescence or the scattering of interfering substances. The method is based on the assumption that the interference can be represented as an analytical function (power series or sum of descending exponentials) of wave lengths.

Ideally the Raman spectrum of a mixture should be a superposition of the spectra of the pure components, the intensities of the bands being proportional to the concentrations of the substances to which they belong. Fortunately, for mixtures of nonpolar or slightly polar compounds, this is true. The usual assumption that there is a superposition of spectra is satisfactory when applied to the range of previous experience; it is, however, only a guide for guessing the performance of mixtures of dissimilar substances.

Several papers bearing on the frequency or intensity changes with concentration have appeared this year. The action of various solvents on the frequencies and particularly on the relative intensities of several tetrahedral molecules (carbon tetrachloride, silicon tetrachloride, and chloroform) was investigated by Fedosow (*22*). The intensities of the lines corresponding to the oscillations perpendicular to the connecting line between the central atom and the corner atom are subject to changes of as much as ±20%. These changes are not correlatable with the magnitude of the dielectric constant or the dipole moment of the solvent.

The spectra of ten mixtures of phosphorus trichloride and phosphorus tribromide were obtained by Theimer (*92*), who observed certain frequency shifts. Bishui and Sanyal (*6*) pointed out the change in the intensity ratio of two lines of ethylene bromide in dilute solutions in methanol, benzene, toluene, and hexane.

Mixtures of dioxane and benzene represent another of the more unfavorable cases for quantitative Raman analysis, and Hanle and Heidenreich reported large deviations in the additivity of the spectra. Richards and Nielsen (*82*) carefully reexamined this system, correcting for the overlapping of the benzene and dioxane bands, and found that the earlier results were erroneous and that the spectra are additive.

Goubeau (*32*) has pointed out an empirical relationship developed by Otting which can be used for correcting the nonadditivity of spectra. Such corrections, however, are not generally useful and whenever an analytical problem is of more than semiquantitative interest the effects of frequency shift and nonlinearity of the intensity-concentration relationship should be investigated.

APPLICATIONS

The variety of applications of analytical Raman spectroscopy continues to be as large as given in last year's review. With the exception of the work of Heigl *et al.* (*39*), all the analyses were performed using procedures previously described.

Luther and Lell (*60, 61*) have devised a semiquantitative scheme whereby type compounds such as olefins, aromatics, alkylcyclopentanes, alkylcyclohexanes, and alkylnaphthalenes can be estimated in light colored lubricating oil fractions. Using these data and Waterman analysis data on the colored portions, more useful information on the composition of petroleum fractions is said to be obtained than by any other analytical method. However, the method for the determination of olefins and aromatics previously cited (*39*) is probably more accurate for these two types alone.

Several additional papers have appeared on the analysis of petroleum fractions (*2, 4, 62, 83, 94*).

A few other applications of interest include the study of the reaction products of the aluminum chloride treatment of 1-dodecene (*65*), the cyclization products from the treatment of paraffin hydrocarbons with platinized carbon (*44*), the products of the contact reaction of cyclohexene and cyclohexadienes with beryllium oxide at 400° C. (*1, 101*), and the reduction product of an acetone–ethyl caprylate mixture (*99*). Addition complexes studied are those of phenol and acetone (*58, 90*), water and nitric acid, and water and sulfuric acid (*10*), various amines and ketones (*91*), and urotropine and acetic acid (*8*).

The pyrolysis products of various rosin oils (*97*) were studied. The compound ζ-fenchene was identified by Raman spectra (*42*), protein hydrolyzates were analyzed (*81*), and the extent of deuterium substitution in deuteriomethyl halides (*5*) was determined.

COLLECTIONS OF SPECTRA

The variety of problems to which any spectral method of analysis can be applied depends to some extent on the size of the "li-

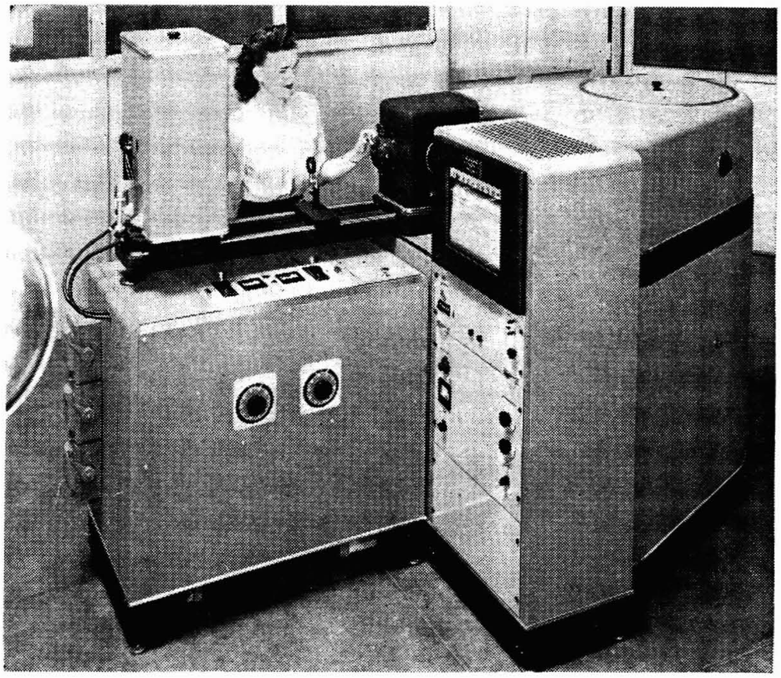

Figure 1. Recording Raman Spectrograph of Lane-Wells Company

brary" of spectra of pure materials available to the analyst. A search of *Chemical Abstracts* will provide the spectra of a great many compounds; however, most of these data are of little use for analytical work because experimental conditions are not given, intensities of lines are not indicated, or relatively impure materials were used.

American Petroleum Institute Project 44 at the National Bureau of Standards is issuing a catalog of Raman spectra to remedy the paucity of good data. The collection now includes 157 spectra (November 1, 1949). Contributions from laboratories which have obtained such data are welcomed, provided the materials examined are relatively pure and the data accurate.

The spectra of 40 terpenes (*9*) and a number of alkyl substituted benzenes (*25*) have also appeared in the literature.

STRUCTURAL STUDIES

A great many papers on the correlation of molecular structure and spectra and on the vibrational analysis of molecules have appeared. Some general review articles are by Goubeau (*32*), who reviewed the progress of the work in Germany during 1939 to 1946, Thompson (*93*), and Lecomte (*57*).

Sheppard (*85*) analyzed the Raman spectral data of a large number of saturated aliphatic hydrocarbons and suggested assignments for the various vibrational frequencies of the CH_2 groups. Sushchinskiĭ (*88*) examined the intensities of several spectral lines of these same compounds and reported an increase in intensity as the CH_2 groups increase for the 2853 and 2908 $cm.^{-1}$ lines, while Shorygin (*86*) studied the intensities of the C═C and CN groups in various molecules. Considerable work has been done on the correlation of molecular structure and spectra, but a great deal still remains to be done before the Raman method can be used as a qualitative and quantitative procedure for type compounds.

Other problems in the vibrational analysis of hydrocarbons which have received attention are: the configuration of cyclohexane (*80*), and the force constants of substituted methylacetylenes (*68*), 1,2-butadiene (*89*), spirohydrocarbons (*3*, *12*), and olefins (*4*).

Additional investigations of interest include: ketene (*36*), methyl fluorides (*20*), hexafluoroethane (*72*), 1,4-dioxane (*79*), acetonitrile (*35*), deuterated ethyl bromides (*40*, *56*), terpene oxides (*59*), aldehydic and ketonic alcohols (*100*), carbonyls (*11*, *48*), dichlorohexafluorocyclobutane (*21*), phosphoryl and thiophosphoryl halides (*18*, *19*), sulfoxylic and thiosulfurous acid derivatives (*29*), metal dialkyls (*23*), tetrahalides (*41*, *98*), pentahalides (*70*), polychloro compounds (*27*), halogenated ethylenic compounds (*47*), and the use of deuterium in the study of vibrational frequencies (*34*).

Various papers have appeared on the approximate computation of vibration frequencies of alcohols, ethers, and amines (*31*, *84*), hydrocarbons (*30*), and other miscellaneous compounds (*24*).

CRYSTAL STRUCTURE

A great deal of work has been done on the study of the Raman spectra of crystals, entirely devoted to crystal structure analysis rather than to chemical analytical problems. The theory, which is at present in a somewhat unsettled state, has been reviewed (*14*, *15*, *43*, *66*, *67*, *73*) and detailed studies of the spectra of a great many crystals have been made. These include: diamond (*50*, *74*, *87*), sodium chloride (*33*, *51*, *69*, *75*), magnesium oxide (*76*), alumina (*53*), topaz (*54*), calcite (*13*, *28*), ammonium chloride (*16*, *49*, *52*), ammonium bromide (*55*), potassium bromide (*33*), lithium and sodium fluorides (*77*), oxalates (*63*), sylvite (*78*), alkali sulfates (*17*), benzene (*26*), and the diphenylbenzenes (*71*).

The spectra of water of crystallization (*64*) and the differences in the spectra of crystals at various temperatures have also been examined (*49*, *52*, *55*, *96*).

Harrand (*37*) has described a new arrangement for obtaining the spectra of crystals.

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ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY

E. J. ROSENBAUM, *Sun Oil Company, Norwood, Pa.*

DURING 1949 few really new developments in the field of ultraviolet absorption spectrophotometry have come to the attention of this reviewer. There has been some progress, but this has been mostly in the direction of the refinement of existing methods and their extension to additional analytical problems. The absorption spectra of a number of compounds have been reported, usually in connection with molecular structure investigations. Of course, each spectrum is potentially the basis of a new analytical application.

A rather detailed description of a method for determining individual C_6, C_7, and C_8 aromatic hydrocarbons in mixtures containing up to six of these components is given by Tunnicliff, Brattain, and Zumwalt (*19*). These authors include in their paper a considerable amount of useful basic information on calibration, test for and removal of interfering absorbers, etc.

Vaughn and Stearn (*21*) carry out the analysis of an isomeric xylene mixture in an unconventional way by making absorption measurements at four wave lengths, calculating two pairs of differences between these measurements, and using these differences with a "working chart" set up during calibration to arrive at the desired analysis. The working chart is a ternary composition diagram based on absorption measurements on a series of mixtures of known composition made up from pure xylene samples. This method eliminates errors due to background absorption which is independent of wave length and it is not limited in accuracy by deviations from Beer's law because the calibration is based on mixture data.

The analysis of mixtures of xylene isomers is also treated by Shostenko and Shtandel (*15*). The spectra of the four butylbenzenes and the three diethylbenzenes are reported by Stair (*16*), who also discusses the analytical usefulness of the various absorption bands. The determination of total aromatics in gasoline is carried out by Yzu and Doblas (*22*), using absorption in the range 260 to 270 mμ.

The problem of correcting for interfering absorption when it is impractical to remove all of the interfering material is treated by Tunnicliff, Rasmussen, and Morse (*20*). By making use of spectrophotometric measurements at a sufficient number of spectral positions, they algebraically obtain and correct for the interfering absorption. This method is reported to give good results when applied to the analysis of monocyclic aromatic hydrocarbons and of naphthalene in samples showing strong interference.

The determination of naphthalene and the methylnaphthalenes has received further attention. A method is described by Armstrong, Grove, Hammick, and Thompson (*1*) who use a Hilger spectrograph and photographic photometry to obtain their analytical data. A similar photographic method is applied by Bryant, Kennedy, and Tanner (*3*) to the determination of naphthalene and its methyl derivatives in Trinidad petroleum. Coggeshall and Glessner (*4*) describe a method of employing the Beckman spectrophotometer which they apply to hydrocarbon mixtures boiling in the kerosene range. An average error of 0.2% based on the total sample is reported for analyses of known mixtures. These authors present a simple empirical method for making a background correction in the determination of naphthalene.

A somewhat unusual application of ultraviolet spectrophotometry is the determination of biphenyl in orange peel by Steyn and Rosselet (*17*). Murray (*13*) determines total phenols in gasolines by extracting them with aqueous alkali and measuring absorption at 290 mμ. LeRosen and Wiley (*10*) extract pyridine and related compounds from hydrocarbon mixtures by means of dilute phosphoric acid and determine their concentration by measurements at 255 mμ. Benzaldehyde, present up to concentration of 0.1% as a contaminant in benzyl alcohol, is determined by Rees and Anderson (*14*) who dissolve the sample in a water-methanol mixture and measure absorption at 283 mμ. A method for determining acetaldehyde in monovinyl acetate at concentrations of the order of 0.001% is given by Jullander and Brune (8). A micromethod for the determination of pentoses (0.5- to 2-mg. samples) is presented by Dunstan and Gillam (6). They dehydrate the sugar with 85% phosphoric acid at 170°, steam-distill the resulting furfuraldehyde, and measure its absorption at 278.5 mμ.

The ultraviolet absorption spectra of nicotine and related compounds have been intensively studied by Swain, Eisner, Woodward, and Brice (18). They point out the analytical applicability of their results. A rapid and precise method for caffeine is described by Ishler, Finucane, and Borker (7). Two papers have appeared on the absorption spectra of steroid derivatives. In one, Mueller (12) describes the near-ultraviolet and visible spectra of steroid–antimony trichloride reaction products. In the other, Djerassi and Ryan (5) report the spectra of steroidal dinitrophenylhydrazones. A method for the determination of penicillin G (benzylpenicillin) which takes advantage of the characteristic absorption of the benzyl group is presented by Levy, Shaw, Parkinson, and Fergus (11). A background correction is made by measuring absorption at a peak (264.5 mμ) and at an adjacent valley (263 mμ) and using the difference for the analysis.

The one instrumental development reported during the past year is the ultraviolet analyzer described by Kivenson, Osmar, and Jones (9). This is not a spectrophotometer but is actually a filter photometer. It is used for the continuous analysis of a flowing styrene-butadiene sample.

In a paper not directed specifically at ultraviolet methods—but parts of which are clearly applicable—Ayres (2) discusses the evaluation of accuracy in photometric analysis.

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X-RAY ABSORPTION

HERMAN A. LIEBHAFSKY, *General Electric Company, Schenectady, N. Y.*

THE present review continues the classification and order laid down in 1948 (7), but it is less theoretical and introduces x-ray fluorescence and safety as new topics. Progress during 1949 in the use of analytical methods based upon x-ray absorption has been steady and greater than the published literature taken alone would indicate.

X-RAY ABSORPTION SPECTROMETRY

Work on the Dow automatic x-ray absorption spectrometer continued during 1949 (3). X-ray tube space current and excitation potential have been stabilized until the statistical fluctuation in the low intensity x-rays is the only evident source of error. The ratemeter circuit for recording the Geiger tube output was improved by incorporating certain recent developments in electrical circuits. Analyses by the method of Glocker and Frohnmayer (see 7, Figure 3) have been carried out for nickel, zinc, selenium, bromine, rubidium, strontium, and mercury. Routine determinations of hydrogen, carbon, nitrogen, oxygen, silicon, sulfur, and chlorine in simple compounds have been made by measuring the absorption of monochromatic x-rays in regions of continuous absorption.

X-RAY ABSORPTIOMETRY WITH POLYCHROMATIC BEAMS

With x-rays, as with radiant energy of longer wave lengths, it is generally true that the precision of an absorptiometric method is significantly increased by making the method comparative—i.e., by commuting in the x-ray beam between the unknown and a suitable standard, such commutation preferably being rapid. This fact was applied in the three recent investigations discussed chronologically herewith.

Kehl and Hart (6) adapted the North American Philips (Geiger counter) x-ray spectrometer to the quantitative determination of sulfur in hydrocarbons. The sample, contained in a glass cell, was placed in the direct beam from an iron-target tube. Six readings were made in each determination, taken alternately on standard and unknown. Variations in the absorption of the hydrocarbon base stock were compensated by use of a calibration curve that correlated transmitted intensity and specific gravity. By the method developed, a technician could obtain in 10 minutes, and to within ±0.025%, the sulfur content of a sample containing from 0 to approximately 3% by weight of that element.

The comparative method has been applied satisfactorily on a laboratory photometer (13) to the identification of pure compounds, the determination of tetraethyllead fluid in gasolines, and the determination of sulfur in crude oils. The determinations of tetraethyllead fluid were carried out on gasolines supplied as unknowns by the Ethyl Corporation. The x-ray results could be expressed in equations such as

$$\text{Equivalent thickness (cm. of Al)} = 0.3815 + 0.0370x \qquad (1)$$
$$\text{Equivalent thickness (cm. of Al)} = 0.3830 + 0.0462x \qquad (2)$$

In these equations, which are of the slope-intercept type, the intercept represents the absorption of the base stock, and the amount of tetraethyllead present is established by the slope. The precision attained was generally comparable with that (estimated to be ±0.02 ml. per gallon) of the chemical method for tetraethyllead fluid. As Equations 1 and 2 indicate, the precision was sufficiently great to warrant consideration of the difference in the base-stock gasolines.

Another of the deviations that can occur with polychromatic beams was pointed out in the foregoing investigation (in *13*, compare Figures 3 and 4).

An important paper by Vollmar, Petterson, and Petruzzelli (*12*) reveals the extensive use being made of the General Electric x-ray photometer in the control testing of petroleum products by an industrial laboratory. The following determinations are discussed: sulfur in hydrocarbon mixtures, tetraethyllead fluid in gasoline, additives (such as metal soaps) in oils, and the metal content of metallo-organic derivatives. Under the operating conditions encountered, the base-stock problem implicit in Equations 1 and 2 could be disposed of in a practical way. Further experience with this photometer was described by Vollmar at a symposium under the auspices of the American Society for Testing Materials (*11*).

X-RAY FLUORESCENCE

It has long been known that characteristic x-rays can be excited through the absorption by matter of x-rays shorter in wave length than the corresponding absorption edges (see *7*, Figure 1). That the identification of these characteristic rays, and a measurement of their intensities, could constitute a method of qualitative and quantitative analysis was clearly appreciated by von Hevesy (*5*), who even mentioned the possibility of investigating liquids in this way. Recently, Friedman and Birks (*4*) have designed a Geiger counter spectrometer for measuring the intensity of these fluorescent x-ray spectra. Here, as in the case of x-ray absorption, improved methods of x-ray detection have operated to make a formerly difficult technique readily accessible to the analytical chemist.

As a welcome consequence of the work of Friedman and Birks, x-ray fluorescence promises to become an important method of chemical analysis. For reasons inherent in the method, it seems likely that the earliest applications may come in the metals industries (*1, 2*). If the expected promise is fulfilled, the method will be discussed more fully in a subsequent review.

SAFETY

Silverman, Elliott, and Greenfield (*10*) have published data to show that the x-rays generated by their electron microscope did, under certain circumstances, lead to dosage rates exceeding the 300 milliroentgens per week considered permissible by the National Bureau of Standards (*8*). All x-ray equipment, particularly that designed for continuous operation, should be surveyed from this point of view. Such surveys made on General Electric x-ray photometers (*7*, Figure 8) running at full load have shown no x-rays detectable externally by an ionization chamber with a threshold of about 1 milliroentgen per hour. The chamber was calibrated with gamma-rays from radium, and the survey was made with the chamber open, so that air was the only absorbing medium (*9*).

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X-RAY DIFFRACTION

H. S. KAUFMAN, *M. W. Kellogg Company, New York, N. Y.*
AND
I. FANKUCHEN, *Polytechnic Institute of Brooklyn, Brooklyn, N. Y.*

The past year has witnessed the continued application of x-ray diffraction to analytical problems. There have been a number of developments in apparatus and technique which have increased the utility of the method as an analytical tool, and it is the purpose of this paper to review some of these developments together with some of the most recent applications. The fundamentals of the technique have been reviewed in a previous paper (*46*).

APPARATUS AND TECHNIQUES

The basic commercial x-ray diffraction units are essentially unchanged. The General Electric XRD-3 unit, announced in 1948, has become available. This unit has provision for film cameras as well as a high-angle Geiger counter spectrometer, and also an attachment for x-ray diffraction fluorescence analysis. This last technique takes advantage of the fact that each element has a characteristic fluorescent radiation. Determination of the wave length and intensity of emitted radiation provides a rapid method of carrying out qualitative and semiquantitative analyses. Cordovi (*24*) has described the technique as carried out with a North American Philips instrument.

A high-angle spectrometer is being produced by North American Philips for attachment to the standard water-cooled diffraction unit. This instrument and the General Electric spectrometer provide a high usable intensity combined with high resolution, and are invaluable aids in analytical work.

Along these lines, though more specialized, is a high-intensity rotating anode x-ray tube, developed by Taylor (*76*), which is capable of operating at 100 ma. in contrast to the normal 20 ma. permitted with a stationary copper anode. This results in much more rapid film exposures. An adjustable focus x-ray tube has been described by Arndt (*6*).

Continued increase in the use of the spectrometer for diffraction work has resulted in greater attention to the fundamental properties and techniques of the system. The measurement of dead time and nonlinearity of Geiger counter response have been discussed (*4*). Attention has been given to the geometrical factors affecting the contours of x-ray diffraction spectrometer maxima (*1*). Arndt (*7*) has described a stable x-ray power supply for use with a spectrometer assembly. McCreery (*53*) has described a mount for powdered specimens which cuts down on errors in intensity through preferred orientation. The introduction of 1 or 2% carbon black into the specimen has also been recommended for this purpose (*19*). In order to increase reading accuracy, a method has been developed for producing a calibrating mark on spectrometer charts (*63*).

There has been continued development of powder cameras and associated techniques. De Wolff (*25*) has described a high-resolution powder camera. Straumanis (*72*) has developed a camera and technique for the precision determination of lattice constants. This technique minimizes errors due to camera radius and film shrinkage. The latter problem has also been discussed by Jellinek (*43*).

A number of high temperature cameras (*27, 31, 49, 69, 82*) have been described, including an apparatus for taking x-ray diffraction diagrams of volatile systems (*39*). Low temperature techniques (*47, 51*) are very useful for the study of materials normally liquid.

Powder method accessories include monochromators (*29, 83*), a simple punch for cylindrical x-ray diffraction cameras (*34*), and a film-to-specimen gage (*79*). Tables of *d* spacings for copper and iron radiation (*74*) and tables of back-reflection *d* spacings for different target materials (*11*) have been published. Stewart and Lutton (*70*) discuss convenient methods for obtaining *d/n* values from x-ray patterns.

An apparatus for preparing capillary tube samples for powder diagrams has been described (*35*). Matthews and McIntosh (*58*) have developed a method of obtaining powder diagrams from single crystals.

The method of presentation of standard x-ray diffraction data for identification purposes is still under discussion. Matthews (*57*) has discussed the use of a punched card code for x-ray powder data. The revised A.S.T.M. identification cards have been announced (*5*) and will be available in normal index card form and in punched card form, if there is sufficient interest.

There have been several developments in the field of small-angle x-ray scattering. Wright and Cole (*84*) have described an improved small-angle camera. A double crystal spectrometer has been used to determine small-angle Bragg reflections (*45*). Warren has discussed the total power of small-angle scattering and its relation to particle size (*80*). Yudowitch (*85*) has described a method of obtaining very soft x-rays (using an aluminum target) for the study of small-angle scattering and particle size. Lund and Vineyard (*52*) have treated the problem of interparticle interference and its effects on small-angle scatter.

Fournier (*30*) has discussed the production and use of very fine x-ray beams (of the order of 2 to 12 μ) for the study of inclusions and microspecimens. Bergmann and Fankuchen (*12*) have modified the Philips microcamera to obtain longer film-to-specimen distances.

The measurement of intensity is fundamental to the utilization of x-ray diffraction for quantitative analysis. The Geiger counter spectrometer is very satisfactory for this purpose, but other methods have also been described. Baenziger's (*9*) method involves the use of a radioactive toner on the x-ray film. The use of electron multiplier tubes (*62*) has also been advocated. The statistical factors affecting the intensity of x-rays diffracted by crystalline powders have been discussed (*3*). A photometer (*21*) for comparison of the intensities of two film patterns has been found very useful for analytical applications.

APPLICATIONS

The speed and reliability of x-ray diffraction have been important factors in its increased use as an analytical tool. Because an x-ray diffraction diagram is characteristic of any given compound, the method is very useful for qualitative determination of compounds present in an unknown. A particularly important application is the identification of components in solid phase reactions. A number of such systems have been investigated, including barium–barium oxide (*50*), uranium-carbon (*28*), and sodium bromide–sodium chloride solid solutions (*61*), and the copper chromite catalyst system (*73*). Jack (*40–42*) has studied the iron, carbon, and nitrogen system and described a new iron carbide. The identification of iron carbides has been considered by Hofer, Cohn, and Peebles (*38*), who used x-ray diffraction and magnetic measurements.

Inorganic compounds characterized by x-ray diffraction include the heavy metal orthovanadates (*59*), manganese dioxide and its modifications (*56*), uranium hexachlorides (*86*), molybdenum oxides (*81*), basic sulfates of lead (*48*), the sulfides and selenides of tungsten (*32*), and the elements actinium to americium (*87*). Boron-coated filaments of molybdenum and tungsten (*75*) have been investigated, as have the corrosion products of natural gas condensate wells (*18*). A spectrometer method has been described for studying rapid phase changes in steels (*37*).

There have been a number of mineralogical studies involving the use of x-ray diffraction: montmorillonite (*33*), chamosite (*16*) (hydrous ferrous silicate), the transformation of montmorillonite minerals to micas (*17*), and the distinction among vermiculite, chlorite, and montmorillonite (*78*). Rustum (*66*) has investigated the decomposition and resynthesis of micas. Micas have also been studied with the aid of a spectrometer (*10*). MacEwan (*55*) has discussed the general problem of clay analysis with the aid of x-ray diffraction.

In the field of organic chemistry there have also been a number of applications of x-ray diffraction analysis. Heavy metal soaps (*77*), aluminum disoaps (Napalm, *60*), commercial soaps (*36*), and surface-active agents (*15*) have been characterized by x-ray methods. Blayden, Gibson, and Riley (*14*) have studied coal by this method. The various penicillins have been identified (*22*) and determined quantitatively (*19*) by x-ray diffraction analysis. Senti and Warner (*68*) have obtained the molecular weight of β-lactoglobulin by x-ray methods. Christ, Burton, and Botty (*20*) have described a method for the identification of small amounts of amino acids separated by chromatography. McCrone (*54*) has continued to present crystallographic data on organic compounds.

The application of small-angle scattering to particle size distribution has been developed further (*80, 85*). Ritter and Erich (*64*) have discussed the determination of pore size from small-angle scatter. Jellinek and Fankuchen (*44*) have investigated the small-angle scatter of alumina gel. Arnell and Barss (*8*) have compared x-ray surface areas with those obtained by nitrogen absorption. Bertaut (*13*) has related Debye-Scherrer line contours to distribution.

X-ray diffraction has also been applied to techniques of microanalysis. Clifton and Smith (*23*) have described a method of microsampling and microanalysis. Rooksby (*65*) discusses the uses of x-ray diffraction in microanalysis.

In addition to the applications that involve identification or qualitative analysis of compounds present, there have also been a number of applications involving quantitative analysis. The ease with which intensities can be obtained on the spectrometer has provided an impetus for the use of this instrument for quantitative work. The evaluation of such factors as x-ray absorption (*2*) and statistical effects on intensity (*4*) serves to increase the precision of these determinations. Duwez and Odell (*26*) have used the spectrometer for the quantitative analysis of mixtures of cubic and monoclinic zirconia. Christ, Barnes, and Williams (*19*) have described a technique for quantitative analysis of organic compounds using a spectrometer, and x-ray diffraction methods have also been used for quantitative analysis of calcium carbonate (*71*) and the allotropic forms of cobalt (*67*). This technique may be expected to become increasingly useful for analytical work.

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EMISSION SPECTROSCOPY

WILLIAM F. MEGGERS, *National Bureau of Standards, Washington, D. C.*

LAST year the writer (*69*) reviewed the principal spectroscopic advances in fundamental research and applications for three postwar years including most of 1948; that review is hereby extended to 1949.

Because the quantum interpretation of atomic spectra contributed so greatly to knowledge of atomic structure and other properties of atoms, basic research in spectroscopy was intensive, and world wide until 1940. Then emphasis in physical research shifted suddenly to ballistics, aerodynamics, electronics, and atomic energy for military purposes. Ten years later this emphasis is unchanged but, thanks mainly to a few faithful prewar spectroscopists, progress has been made in standard wave lengths, term analysis, Zeeman effect, isotope shifts, hyperfine structure, and new elements.

STANDARD WAVE LENGTHS

The chain-reacting uranium piles, primarily constructed for making atomic bombs, have yielded artificial elements that are of inestimable value to science—for example, the abundance of neutrons from $^{35}_{92}U$ fission makes possible plentiful production of pure $^{198}_{80}Hg$ by transmuting $^{97}_{79}Au$. Spectroscopic light sources containing this artificial isotope of mercury emit spectral lines of greater sharpness than any found in nature and the wave length of the green line of $^{198}_{80}$Hg has been proposed (*70*) as the ultimate standard of length. Preliminary measurements of the wave lengths of the green and yellow lines of $^{198}_{80}$Hg have been reported (*82*) by the National Bureau of Standards in Washington, the National Physical Laboratory near London, and the International Bureau of Weights and Measures near Paris. The values in International Ångströms are:

N.B.S.	N.P.L.	I.B.W.M.	Mean
5460.7532	5460.7531	5460.7533	5460.7532
5769.5983	5769.5985	5769.5986	5769.5985
5790.6628	5790.6628	5790.6630	5790.6629

This is probably the first time in history when three different nations have come so close to nearly perfect agreement.

The only other contribution in recent years to precise measurements of wave lengths by interferometer technique is by Burns and Sullivan (*17*), who observed the spectrum of nickel in the vacuum arc from 2173 to 8968 A. They also reported some wave lengths of cobalt, titanium, manganese, magnesium, chromium, and aluminum lines observed incidentally as impurities in nickel.

TERM ANALYSIS

Although hydrogen emits the simplest of all spectra, it has been the subject of more than 1000 spectroscopic papers. The spectrum of hydrogen has a fine structure which, according to the Dirac wave equation for an electron moving in a Coulomb field, is due to the combined effects of relativistic variation of mass with velocity and spin-orbit coupling. The ground state is 1*s* $^2\mathrm{S}_{1/2}$ and the first excited states are 2*s* $^2\mathrm{S}_{1/2}$ and 2*p* $^2\mathrm{P}^\circ_{1/2,\,3/2}$. According to the Dirac theory the 2*s* $^2\mathrm{S}_{1/2}$ state coincides exactly with 2*p* $^2\mathrm{P}^\circ_{1/2}$. Experimental attempts to confirm this through a study of the Balmer lines have been frustrated by the large Doppler width of the lines in comparison with the splitting of the $n = 2$ states, which is about 0.36 cm.$^{-1}$ for 2 $^2\mathrm{P}_{1/2}$ − 2 $^2\mathrm{P}_{3/2}$. However, the great wartime advances in microwave techniques provided new methods for a study of the $n = 2$ states of the hydrogen atom. Such a study by Lamb and Retherford (*60*) indicated clearly that the 2 $^2\mathrm{S}_{1/2}$ state is higher than the 2 $^2\mathrm{P}_{1/2}$ state by about 1062 megacycles per second, or 0.035 cm.$^{-1}$

This result inspired several conventional spectroscopists to look for an analogous shift of S-levels in the hydrogenlike spectrum of ionized helium, and such investigations have been reported by Fowles (*29*), Kopfermann and Paul (*54*), and Hirschberg and Mack (*41*). Microwave techniques were applied to this problem by Skinner and Lamb (*94*).

Notable extensions of the principal series have been observed in the absorption spectra of sodium, potassium, rubidium, and cesium. Thus, McNally (*63*) measured the sodium series to $3^2\mathrm{S} - 52^2\mathrm{P}^\circ$, while Thackeray (*98*) observed it to $79^2\mathrm{P}^\circ$. Kratz (*56*) reported observations on the principal series of potassium to $79^2\mathrm{P}^\circ$, rubidium to $77^2\mathrm{P}^\circ$, and cesium to $73^2\mathrm{P}^\circ$. McNally *et al.* (*66*) observed the cesium series to the 62nd member. These long, regular series have been extrapolated to limits from which five- or six-figure ionization potentials can be derived. In addition to these principal series, Kratz and Mack (*57*) have observed in long-path absorption "forbidden" lines of type $2^2\mathrm{S} - n^2\mathrm{D}$ to $13^2\mathrm{D}$ for potassium, $21^2\mathrm{D}$ for cesium. A path of 24 meters of rubidium vapor at 278° C. showed $5^2\mathrm{S} - nl$ lines to $54^2\mathrm{D}$ and also from $29^2\mathrm{F}^\circ$ to $50^2\mathrm{F}^\circ$ and from 29 *q* to 47 *q* where *q* is the *l* value of nonpenetrating electrons with $l > 3$. With much difficulty Vancouleurs (*102*) extended observation of potassium subordinate series in emission from $4^2\mathrm{P}^\circ - 11^2\mathrm{D}$ to $16^2\mathrm{D}$ and $4^2\mathrm{P}^\circ - 13^2\mathrm{S}$ to $14^2\mathrm{S}$.

By observing the hollow-cathode spectrum of cadmium over the wave-length range 600 to 6500 A., Shenstone and Pittinger (*90*) were able to refine and extend data and analysis for the first three spectra of cadmium and calculate 37.6 electron volts as the third ionization potential.

Recent improvements in infrared spectrometers and detectors have been applied to observations of the spectra of noble gases beyond the long-wave limit accessible to photography. Many new lines between 1.2 and 2.2μ were observed for argon, krypton, and xenon by Sittner and Peck (*93*), and by Humphreys (*44*) who also observed neon and classified practically all new lines as combinations of known and new levels, particularly those arising from the $s^2p^5f^1$ configuration.

Notable progress in the description and analysis of complex atomic spectra can be reported for chromium, manganese, tantalum, technetium, uranium, neon, argon, krypton, and xenon, although the details are mostly still unpublished. Kiess (*49*) remeasured arc, spark, and Zeeman spectrograms of chromium and has practically completed the structural analysis of Cr I and Cr II spectra by finding atomic energy levels that account for ca. 2800 Cr I and 1400 Cr II lines. The Cr III spectrum was investigated by Moore (*76*), who found 41 terms that accounted for 580 lines; he is also investigating the spectrum of trebly ionized chromium.

Completely new measurements of arc, spark, and Zeeman spectra of manganese permitted Catalán (*19*) finally to complete the analysis of Mn I which he began in 1921. Catalán's discovery in chromium and manganese spectra of terms with five or six levels each was the catalyst for the interpretation of complex spectra and development of quantum theory. Catalán first proposed that a group of lines resulting from the combination of two complex terms be called "a multiplet"; he has now recognized more than 400 such multiplets in the Mn I spectrum comprising about 2000 lines. Catalán is also working on the Mn III spectrum, while Curtis (*22*) is extending the analysis of Mn II that he published a dozen years ago. The wave lengths and relative intensities of more than 2000 lines characteristic of the first two spectra of technetium (a uranium-fission product) have been determined by Meggers and Scribner (*71*), who have also classified the stronger lines of Tc I and Tc II spectra as combinations of identified sextet and septet terms, respectively. The ground state of $_{43}$Tc atoms is established as $(4d^55s^2)^6\mathrm{S}_{2\frac{1}{2}}$ but the lowest energy level of $(4d^65s^1)^6\mathrm{D}$ is only 2572.9 cm.$^{-1}$ higher.

The wave lengths of several thousand lines characteristic of promethium (another uranium-fission product) have been measured by Meggers and Scribner (*72*) but the data are still inadequate for the differentiation of successive spectra or the analysis of spectral structure. They confirm, however, the discovery of a new element of rare-earth type—viz., $_{61}$Pm. The arc and spark spectra of $_{75}$Re have been photographed with pure metal electrodes to provide complete and accurate data for the final analysis of Re I and Re II spectra. The observed wave lengths range from 2000 to 12,000 A. Investigation of the spectra of tantalum has been in progress for many years; a preliminary report on terms and magnetic splitting factors established for Ta II was recently sent to press by Kiess *et al.* (*50*). Progress in the analysis of the second spectrum of $_{92}$U, supported by Zeeman effect, has been reported from the Massachusetts Institute of Technology (*65*) and from the Zeeman Laboratory (*7*). In the latter a search for connections between two independent groups of classified lines failed but added 17 levels to one group and 63 to the other, thereby increasing the number of classified lines by 392 to a total of 743. The relative energy of levels from $5f^47s^1$ and $5f^37s^2$ is still unknown.

The spectra of the heavy elements, protactinium, neptunium, plutonium, and americium, like those of thorium and uranium, are characterized by very great complexity. Consequently, descriptions of these spectra to be adequate for term analysis and assignment of electron configurations must include accurately measured wave lengths, relative intensities, excitation criteria for thousands of lines, and resolved Zeeman patterns for the stronger ones. Because considerable effort and amounts of sample are required, it will be some time before such descriptions are available.

It may be presumed that the production and purification of these ponderous particles will be controlled by spectrochemical analysis. For this purpose it is sufficient to have approximate wave lengths and calibrated intensities of prominent lines in a convenient spectral region. Tomkins and Fred (*100*) have published such data for 263 lines of protactinium from 2640.3 to 4371.7 A., 114 lines of neptunium from 2655.0 to 4363.8 A., 220 lines of plutonium from 2677.0 to 4358.1 A., and 227 lines of americium from 2661.6 to 4374.9 A. Their line intensities are proportional to the reciprocal of the limiting dilutions at which they could be recorded photographically with a copper spark source and a 3-meter grating spectrograph. A striking resemblance of americium and europium spectra (both elements have a few lines of outstanding intensity) suggests by analogy that americium has in its normal state seven $5f$ electrons and two $7s$ electrons.

Relatively little has been done recently on atomic spectra beyond the IIIrd. (Edlén is building a new physical laboratory in Sweden, and in the United States all prewar vacuum spectrographs are still idle except one at Princeton University.) In India three spectra of multiple ionized iodine have been investigated: I III (*59*), I VI (*58*), and I VII (*27*).

Most of the results for spectral terms of light elements reviewed above will be found in Volume I of "Atomic Energy Levels" (*74*), which contains data on ionization potentials, electron configurations, spectral terms, quantum numbers, and magnetic splitting factors (where known) for 206 spectra characteristic of the first 23 atomic numbers, $_{1}$H to $_{23}$V, inclusive. The remainder (and more) will appear in future volumes of this work, Volume II of which (now in press or preparation) will contain similar data on all known spectra of at least 18 atomic numbers, $_{24}$Cr to $_{41}$Nb, inclusive.

ZEEMAN EFFECT

Because the Zeeman effect is the most effective aid in the analysis and interpretation of spectra, further observations of line splitting and polarization in magnetic fields are urgently needed. Such observations have been reported recently for Au I and Au II by Green and Maxwell (*34*) and for U I and U II by Bosch and Berg (*8*). In uranium magnetic splitting factors (g-values) are given for 37 U I levels and for 82 U II levels. In both gold and uranium departures from LS-coupling of the optical electrons are indicated by the observed magnetic splitting of spectral lines. The paper by Kiess and Shortley (*51*) on the Zeeman effect for nitrogen and oxygen is important not only because these are the lightest elements for which magnetic splitting factors have been determined, but also because it shows for the first time how g-values are properly derived from unsymmetrical Zeeman patterns exhibiting partial Paschen-Back effect, which is all too common in many spectra.

The diamagnetic quadratic Zeeman effect in the principal series of K I, Rb I, and Cs I spectra has been investigated by Harting and Klinkenberg (*37*). This effect originates in atomic diamagnetism and is therefore proportional to the mean square of the radius of the electron orbit, or to the fourth power of the effective quantum number. It results in a quadratic shift of high levels, giving rise to a quadratic displacement of the magnetic components to shorter wave lengths. Measurements were made on wave lengths of absorption lines to effective quantum numbers exceeding 30, in magnetic fields of 22,600 oersteds, and the observed displacements were found to agree closely with those predicted.

Zeeman spectrograms made in the Spectroscopy Laboratory at the Massachusetts Institute of Technology have recently been measured for the derivation of g-values of the Mn I and Mn II terms (*19*) and of Fe II terms (*104*). After 10 years of regretted interruption, the M.I.T. Zeeman-effect program was resumed in 1949 when superior Zeeman spectrograms were obtained with arcs of $_{24}$Cr, $_{25}$Mn, $_{44}$Ru, $_{72}$Hf, $_{73}$Ta, $_{75}$Re, $_{76}$Os, and $_{77}$Ir in magnetic fields of 80,000 to 90,000 oersteds. This practically completes the observation of Zeeman effect for most of the common metals, but a great deal still remains to be done with rare earths, whereas the investigation of Zeeman effect for unstable elements created either by transmutation or by fission has only been suggested. There are good prospects that radiations from extremely rare and radioactive elements will be investigated in magnetic fields because improvements in enclosed light sources permit observations with minute samples that may be recovered for other purposes.

FORBIDDEN LINES

Atomic spectra almost universally obey simple selection rules governing transitions between atomic energy levels, but from time to time "forbidden lines" are detected in various sources. Thus, three forbidden O I lines (5577, 6300, and 6363 A.) long ago detected in the spectrum of the aurora borealis have recently been identified by Bowen (*9*) and by Cabannes and Dufay (*18*) as faint absorption lines in the solar spectrum.

Six diffuse lines (2819 to 3005 A.) attributed to Cd F are now explained (*81*) as Cd I forbidden transitions forced by ionic electric fields in a high-current arc. This example shows that it is easy to mistake asymmetrical atomic lines for band heads as well as vice versa.

A suggestion that forbidden lines in the spectra of $_{30}$Zn, $_{48}$Cd, and $_{80}$Hg arise from the magnetic moments of isotopic nuclei with odd-mass numbers was tested for $_{48}$Cd by Deloume and Holmes (*23*), who compared the intensities of 3320 A. ($5^3P_0^\circ - 5^1S_0$) and 3141 A. ($5^3P_2^\circ - 5^1S_0$) emitted by an electrodeless discharge in natural cadmium vapor containing 23% $^{111}_{48}$Cd, with the same lines emitted by cadmium vapor enriched with 64.5% $^{111}_{48}$Cd, and reported that the intensities of the forbidden lines were indeed proportional to the abundance of $^{111}_{48}$Cd.

ISOTOPE SHIFTS

The spectra of hydrogen, deuterium, and tritium differ slightly and progressively, primarily because the isotopic nuclei have different masses. Indeed, this fact was the inspiration for the discovery of heavy hydrogen. Later studies in other spectra made it appear that factors other than mass contributed to the observed displacements, but up to the present time no satisfactory theory of isotopic shifts has been developed. Last year Andrew and Carter (*2*) reported measurements of the isotope shift of the red line (6678 A.) of $^{3}_{4}$He relative to $^{4}_{4}$He in agreement with the theory of Hughes and Eckart. Other measurements on the same and on other $_{4}$He lines by Bradley and Kuhn (*10*), by Manning (*68*), and by Fred *et al.* (*31*) are neither in close agreement with each other nor with the theory. Preliminary results on the line shifts of $^{20}_{10}$Ne relative to $^{22}_{10}$Ne have been reported by Murakawa and Suwa (*78*) for nine lines (3319.8 to 3727.1 A.) of the Ne II spectrum. It appears that lines of the doublet system show larger isotope shifts than those of the quartet system.

Because the isotope shift of Ba I lines is too small to observe by usual methods, Kopfermann and Wessel (*55*) studied the resonance line 5536 A. in absorption of an atomic beam illuminated by light from a hollow cathode, and concluded that two barium isotopes differing by two units of mass show line shifts of 0.012 ± 0.002 $cm.^{-1}$

Isotope shifts in the spectrum of $_{62}$Sm have been carefully measured by Brix and Kopfermann (*11, 13*) and by Brix (*12*). Comparison (*11*) with earlier results for $_{60}$Nd leads to the discovery that isotopes with equal numbers of neutrons (but different numbers of protons) show the same isotope shifts of spectral lines.

Brix (*12*) measured $^{152}_{62}$Sm − $^{154}_{62}$Sm isotope shifts of 80 lines of Sm I between 5044 and 7132 A. The lines were sorted into several groups according to magnitude and sign of shift, which in turn were correlated with atomic energy levels. This analysis of isotope shifts confirmed almost completely the term analysis of Albertson, and added four new levels. Furthermore, it was possible with isotope shifts to group many of the observed levels into terms and assign the proper electron configuration. This is the first instance in which isotope shifts have made a real contribution to the structural analysis of a complex spectrum.

Isotope shifts among hundreds of lines (2500 to 4800 A.) emitted by uranium isotopes $^{233}_{92}$U, $^{235}_{92}$U, and $^{238}_{92}$U were observed by Burkhart *et al.* (*16*), who concluded that any line shifted from ${}^{238}_{92}U$ to ${}^{235}_{92}U$ was also shifted from ${}^{235}_{92}U$ to ${}^{233}_{92}U$, that the direction of shift of lighter isotopes is generally toward shorter waves, and that there appeared to be no correlation between the actual shifts and the mass numbers. When McNally (*64*) compared the isotope shifts in uranium spectra with U I and U II energy levels and electron configurations, he found a simple shift arising from an interaction of a configuration with a nucleus, a complex shift arising from perturbations between close-lying levels, and the largest shifts involved configurations containing s^2 electrons, indicating an additive type of interaction for the *s*-electrons and the nuclei.

So long as there is no adequate theory or general understanding of isotope shifts, it is perfectly proper to grope for correlations and generalizations. How else can one hope to stumble upon the basic laws of nuclear physics?

HYPERFINE STRUCTURE

When optical electrons interact with atomic nuclei that possess magnetic momenta, the spectral lines are split into hyperfine components which, if resolved and measured, lead to quantitative determination of the mechanical, magnetic, and quadrupole moments of the nuclei. There is no ambiguity about the theory and interpretation of hyperfine structure; the only difficulty is an understanding of the manner in which the observed properties of nuclei can be compounded from the known properties of the elementary particles that comprise the nuclei. In the meantime rapid progress is being made in the accumulation of data on properties of nuclei. From grating spectrograms of P III and P IV lines exhibiting hyperfine structure Crawford and Levinson (*21*) have deduced for ${}^{31}_{15}P$ a spin of $^{1}/_{2}(h/2\pi)$ and a magnetic moment of 1.15 nuclear magnetons. The hyperfine structure of Al II lines (6226, 6231, and 6243 A.) was resolved with interferometers by Suwa (*97*) who found good agreement with theory even when the fine structure separations are small.

The *hfs* of an 80% enriched isotope ${}^{125}_{52}Te$ was found by Fowles (*30*) to consist of doublets with intensity ratio 3 to 1, indicating a spin of $^{1}/_{2}$. Enriched samples of ${}^{77}_{34}Se$ and ${}^{123}_{62}Te$ in the hands of Mack and Arroe (*62*) indicated for the former a spin greater than $^{1}/_{2}$, and for the latter $^{1}/_{2}$. The electron structure of ${}_{52}Te$ is not sufficiently well known to determine the nuclear magnetic moment, but the *hfs* splitting of the two isotopes is found to be identical except for a scale factor which yields the tentative value $\mu_{125}/\mu_{127} = 1.208$ for the ratio of the moments. For an enriched sample of ${}^{91}_{40}Zr$, Arroe and Mack (*4*) found a spin of $^{5}/_{2}$.

No hyperfine structure could be detected in enriched samples of ${}^{57}_{26}Fe$ either by Gurevitch and Teasdale (*35*) or by Brossel (*15*). It is pointed out (*35*) that many elements with odd atomic mass but even nuclear charge have no measurable magnetic moment.

The hyperfine structure of electromagnetically separated isotopes of ${}^{21}_{10}Ne$ and ${}^{83}_{36}Kr$ has been excited in Geissler tubes and resolved with Fabry-Perot interferometers by Koch and Rasmussen (*53*). For ${}^{83}_{36}Kr$ a spin moment of $^{9}/_{2}$ is accurately determined from the interval rule, and by comparing intensities of *hfs* components of Ne I lines 5852 and 6266 A., whose *hfs* arises from splitting of lower and higher energy levels, respectively, a spin of $^{3}/_{2}$ (or greater) and a negative magnetic moment was indicated for ${}^{21}_{10}Ne$. The *hfs* of two magnesium lines (5167 and 2796 A.) excited in an atomic beam source was resolved (*48*) with a Fabry-Perot interferometer; the results show that ${}^{25}_{12}Mg$ has a spin moment of $^{5}/_{2}$ and a negative magnetic moment equal to -0.95 ± 0.05 nuclear magneton.

Spectroscopic *hfs* studies of the resonance lines of Ba II from concentrated samples of ${}^{135}_{56}Ba$ and ${}^{137}_{56}Ba$ show, according to Arroe (*3*), that both isotopes have the spin $^{3}/_{2}$ and that the splittings in the latter are 12% larger than in the former.

Many other determinations of magnetic moments and gyromagnetic ratios derived from magnetic resonance or from microwave measurements could be included here if this review were not restricted to conventional spectroscopy.

SPECTROCHEMICAL ANALYSIS

The most colossal contribution to spectrochemical analysis in recent years is the "Atlas der Restlinien" in three magnificent volumes published by Gatterer and Junkes (*33*) in the years 1937, 1945, and 1949, respectively. These volumes contain the most trustworthy tables and photographic prints now available for arc and spark spectra of 73 elements.

Volume 1 presents the spectra of 30 common elements (Ag, Al, Ba, Bi, C, Ca, Cd, Co, Cr, Cu, Mg, Mn, Mo, Ni, Pb, Rb, Si, Sn, Sr, Ti, Tl, V, W, and Zn); Volume 2, the spectra of 18 rare-earth and related elements (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sc, Sm, Tb, Th, Tm, Y, Yb, Zr); Volume 3, the spectra of 19 rare metals and several metalloids (Au, As, Be, Ga, Ge, Hf, In, Ir, Nb, Os, Pd, Pt, Re, Rh, Ru, Sb, Ta, Te, U, and Cl, Br, I, S, Se, and Hg). These wave-length tables extend usually from 2200 to 7000 A. for arc spectra and 2200 to 4500 A. for spark spectra; they omit many faint lines but give for the rare earths more than 9000 new lines.

This monumental atlas was personally prepared by the authors with painstaking care; it was designed for spectrochemical identification and it will be extremely useful to spectrographers the world over.

Another contribution of inestimable value to spectrochemical analysis is the compilation of "Atomic Energy Levels" (*74*). In addition to all known facts concerning atomic energy levels these volumes contain references to all known sources of classified spectral lines. In these volumes wave lengths and intensities are omitted because they (together with excitation potentials) are given in supplementary tables of multiplets (*75*) including practically all lines of interest to applied spectroscopy.

The only other book published since 1948 that deserves mention in this review is "Practical Spectroscopy" by Harrison, Lord, and Loofbourow (*36*).

The scope of this article does not permit listing all the papers on applied spectroscopy recently published; this will occur in Part III of "An Index to the Literature on Spectrochemical Analysis, 1945–1950," by Scribner and Meggers (*87*). In this article only a few selections from the recent papers are reviewed to illustrate applications or call attention to significant developments.

Continuing efforts to improve spectroscopic light sources are noted: a magnetic stabilizer for direct current arcs (*20*), an air-interrupter type light source (*24*), an improved spectrographic power source (*25*), and a direct current arc source and automatic controller (*28*). Huldt (*43*) made a scientific study of the alternating current arc between carbon electrodes saturated with solutions of barium or copper and sodium, and determined arc temperatures from intensities of copper and barium lines whose transition probabilities were known. Temperatures of 4380° and 5260° K. were obtained from barium and copper lines, respectively, but when sodium was present these temperatures were reduced to 3890° and 4580° K. The partial pressure of free electrons as calculated from the relative intensities of Ba I and Ba II lines was found to be of the order of 10^{-5} and 10^{-4} atmosphere at the barium and copper temperatures, respectively. It was demonstrated that line-intensity changes caused by sodium can be calculated if temperatures and electron pressure have been measured.

Studies of the variations of line-intensity ratios caused by the additions of extraneous substances have been reported by Langstroth and Andrychuk (*61*) and by Brode and Timma (*14*). The former studied (in the condensed spark) variations of 86 line pairs of 19 pairs of elements as a function of the test elements, the extraneous elements, the line pair, and presence or absence of a buffer. The variations bore no apparent relation to any physical properties of the added elements but were, in general, smallest for line pairs having most nearly equal excitation potentials. The latter studied extraneous-element effects in the direct current arc, and reported a correlation of line-intensity variations with boiling points and excitation potentials of the elements. The moral of these two studies is: Do not ignore the natural excitation characteristics of spectral lines.

Although the gas flame is not regarded as a general-purpose light source, the flame photometer appears suddenly to have come in vogue for the quantitative determination of nitrogen, potassium, and calcium in almost anything (*6, 77, 80, 83–85, 88, 89, 101*).

Because of the magnitude of the metallurgical industry and because the spectra of metals are most conveniently excited and observed, spectrochemical analysis of alloys continues to produce papers in large numbers. Of special interest are microspectrochemistry of clad aluminum alloys (*47*) and spectrographic examination of localized heterogeneity in metals (*52, 79*). Spectrochemical analysis is also employed successfully and extensively in the ceramics industry—for example, for improvement of analytical control for silica brick (*86*), for analysis of silica refractories (*40, 46, 96*) and for determination of trace elements in silica (*45*). One of the most unusual applications of spectrographic analysis is to the chemical topography of the trace elements in the brain of man (*103*), which purports to prove that sections of the brain that differ in morphological structure and physiological function possess a different chemical composition of the trace elements: silver, aluminum, bismuth, chromium, copper, manganese, molybdenum, nickel, lead, silicon, tin, titanium, vanadium, and zinc.

That spectrochemical analysis is not necessarily restricted to the detection and determination of metals is demonstrated by the spectrochemistry of the metalloids, fluorine, chlorine, bromine, iodine, sulfur, and selenium (*32*). Chemically the rare-earth elements are notoriously identical and inseparable but spectrographically they are, in principle, as easily distinguished and determined as any other elements. It is, therefore, not surprising to find papers published on the spectrographic determination of rare earth elements (and yttrium) in uranium compounds (*42, 91*), quantitative spectrographic analysis of the rare-earth elements (determination of 8 to 100% of yttrium and gadolinium in complex mixtures, *26*), and analysis of rare earth oxides by means of emission spectra (persistent lines in arc spectra of cerium, dysprosium, erbium, europium, gadolinium, holmium, lanthanum, lutetium, neodymium, praseodymium, samarium, terbium, thulium, ytterbium, and yttrium, *95*).

Early in the development of the art of spectrochemical analysis it was modestly claimed that trace elements and minor constituents (say less than 1%) could be more accurately determined by this method than by conventional chemical (gravimetric) methods. The spectrographic methods were gradually refined by improving light sources and removing or reducing errors of observations, so that before long they were yielding highly satisfactory results for concentrations up to 10%, or even higher. Recent applications to rare earths (*26*), to high-cobalt alloys (*92*), and to high-alloy steels (*38, 39, 67*) prove that spectrochemical methods now encompass the entire range from 0 to 100% without yielding much, if anything, to the conventional chemical methods.

Three significant advances have been made in the last year in connection with the spectrochemical analysis of high alloys. The first is the development of source conditions which, in combination with the latest direct-reading apparatus, allow a precision of analysis for high percentage constituents of from 0.3 to 0.4% of the quantity being measured, and this by means of a single determination. The second is the accomplishment of this high precision under excitation conditions which provide complete independence in the determination of one element from changes in the concentrations of the other elements. The third is the development of rigorous calculating methods which make possible the rapid analysis of all constituents, including those of high percentage, in an alloy system. Net result is high speed, direct-reading methods of analysis which can compete in accuracy, for all metallic constituents, with routine chemical methods (*38*).

Fifteen years ago the time required for a spectrographic determination was of the order of 10 to 20 minutes, but by increasing the speed of photographic processing this was reduced to an absolute minimum of 2 or 3 minutes. The next step was to eliminate photographic recording and substitute photoelectric direct reading which made possible the quantitative determination of a dozen or more elements, all in 30 seconds or less. Is this the end of our race with time? Apparently not. Recent papers (*1, 5*) discuss the orthicon spectrograph. "This instrument consists essentially of a television pickup tube, the RCA image orthicon, whose photosensitive surface is placed in the focal plane of a large-aperture spectrograph. A curve of intensity versus wave length (0.45 to 1.0μ), at any chosen instant or succession of instants, appears on the screen of an oscilloscope whose scans are synchronized with those of the orthicon." With this new instrument spectra of an exploding bomb were obtained at the rate of one every millisecond!

The scope of spectrochemical analysis has been enlarged by new elements that may become abundant either as fission products (${}_{43}$Tc, ${}_{61}$Pm) or through transmutations (${}_{89}$Ac, ${}_{91}$Pa, ${}_{93}$Np, ${}_{94}$Pu, ${}_{95}$Am, ${}_{96}$Cm). Indeed, it may be assumed that in addition to having served as proof of discovery the emission spectra of these elements have already been employed in laboratories for detection and quantitative determination. It is no secret that in the copper spark the most intense lines have been reported to have the following wave lengths:

${}_{43}$Tc	4297.2, 4261.9, 4095.3, 4088.4, 4031.3 A. (*99*)
${}_{61}$Pm	4086.0, 3980.6, 3910.4, 3711.7 A. (*99*)
${}_{91}$Pa	3957.8, 3054.6, 3053.5 A. (*100*)
${}_{93}$Np	4290.9, 3829.2 A. (*100*)
${}_{94}$Pu	3989.7, 3907.1 A. (*100*)
${}_{95}$Am	2969.4, 2832.3 A. (*100*)

These elements are not now spectroscopically detectable in the earth's crust but they (especially fission products) may very well be determinable in densely populated regions after a sufficient number of atomic bombs have been exploded.

Finally, to avoid leaving the impression that spectroscopy is applicable only to terrestrial substances it must be emphasized that it is the chief tool of astrophysicists, in whose hands it has revealed the composition, temperatures, pressures, motions, etc., of celestial objects and properties of interstellar matter. A bibliography of quantitative spectral analysis of the sun, solar corona, stars, comets, and nebulae can be found in the 1948 *Transactions of the International Astronomical Union* (*73*).

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MASS SPECTROMETRY

MARTIN SHEPHERD AND JOHN A. HIPPLE, *National Bureau of Standards, Washington 25, D. C.*

DURING the past year, mass spectrometry has advanced partly in the interesting though somewhat confusing manner characteristic of the emphatic but uncertain equestrian who mounted his horse and rode off in all directions at once. So we find, in various stages of discussion, planning, and construction huge spectrometers much greater than existing instruments, and little spectrometers much smaller than the present conventional size. The large spectrometers with crossed fields are intended for the measurement of fundamental constants, and a resolution of about 25,000 was expected of one of them before its final completion was abandoned. The smaller ones, of the negative-ion or the magnetic-resonance types, may, in time, prove to be the poor man's analytical mass spectrometer, for their resolution is good enough for many analytical applications. A continuous recording instrument of limited range has appeared.

Analytical application, as well as instrument design, has branched out. In development work, rather more effort has been devoted to the analysis of liquids or their heavy vapors than to gases. Furthermore, the simple gaseous mixtures have not attracted the attention accorded deuterated compounds and isotopes. Thus the field is becoming more interesting and also more diffuse. Before the diffusion has mixed everything too well, the American Society for Testing Materials, through its Committee D-3 on Gaseous Fuels, is standardizing procedures for the analysis of the simpler gaseous mixtures.

ANALYTICAL APPLICATIONS

Hindin, Grosse, and Kirshenbaum have applied the "internal-standard" method to mass spectrometric analysis, and so opened a promising line of development (*25*, *34*). In conventional analysis, the element sought is usually quantitatively converted to a specific compound of suitable properties, and this is isolated in pure form and quantitatively measured. In the internal-standard method, the element sought is determined relative to another not originally present but added in known amount. For the spectrometric approach, if the sample is not gaseous, it is made to react to convert the element sought quantitatively to the gaseous state, in which the internal standard is present, and a portion of the product is withdrawn for analysis. The ratio of the unknown element to the known internal standard is constant and discloses the amount of the unknown. Thus no quantitative recovery of the reaction product is necessary. In this manner nitrogen has been easily determined in organic materials; and isotopic analysis for oxygen, carbon, and nitrogen in organic materials has been described.

A considerable amount of work has been done in developing modifications of the inlet system and special techniques for the analysis of water, alcohols, and oxygenated compounds. There are some interesting reports in preparation, and three important papers have been published by Gifford, Rock, and Comaford (*24*), Langer and Fox (*50*), and Thomas and Seyfried (*76*). The greatest problem associated with analysis of this type was adsorption in the inlet system, which contributed gorgeous errors. Placing the leak virtually within the ionizing chamber and heating the system and trap, together with proper equilibration techniques, solved the difficult problem. Thomas and Seyfried also used an internal standard (C_6H_6), and reported the analysis of oxygenated compounds containing five or fewer carbons to ±2% or better, water to 1%. Compounds analyzed included alcohols, aldehydes, esters, ethers, and acids. Gifford, Rock, and Comaford also reported the analysis of dilute aqueous solutions of oxygenated compounds.

In addition to the two important developments discussed in the above two paragraphs, some difficult mixtures have tempted several workers. Honig (*40*) has reported the analytical technique used in the complicated analysis of hydrocarbons enriched in C^{13}, including $C\cdot CCC$, $CC\cdot CC$, $C\cdot\overset{C}{C}C$, and $C\overset{C}{C}\cdot C$. Mohler and Dibeler (*60*) have discussed the mass spectrometric analysis of C_2H_2, C_2D_2, and C_2DH. Brinton and Blacet (*11*) have studied a mixture of deuterated aldehydes. Mohler, Bloom, Lengel, and Wise have shown what can be done in the analysis of fluorocarbons with seven or fewer carbons (*58*). The analysis of gaseous hydrocarbons using both the mass and infrared spectrometers is described by Milsom, Jacoby, and Rescorla (*57*). The method of calculation combines data from both spectrometers in a set of $2n$ equations in n variables, solved by the reciprocal matrix method. The infrared spectrometer neatly assists the mass spectrometer in the determination of compounds with many isomers.

APPLICATIONS TO CHEMISTRY AND PHYSICS

Mass spectrometric procedures previously developed have been used for various purposes. Bailey and Van Meter (*2*) corrected the Dumas microanalysis by the mass spectrometer. Farmer and Brown (*21*) studied the deterioration of methane-filled Geiger-Müller counters. Robertson (*68*) used the mass spectrometer in studying the pyrolysis of methane, ethane, and n-butane on a platinum filament. Madorsky, Straus, Thompson, and Williamson (*55*) studied the pyrolysis of polyisobutene, etc., in like manner. Dibeler and Taylor (*14*) studied the deuterium exchange, isomerization, and hydrogenation of the n-butenes. Weller and Friedel (*78*) determined the isomer distribution in hydrocarbons from the Fischer-Tropsch process. Kistemaker (*49*) has reviewed some of the chemical applications of the mass spectrometer.

Probably the outstanding event this year in the measurement of packing fractions was the announcement of the results of Roberts and Nier (*67*) obtained with the electrometric method. These results indicate a precision comparable with the best yet attained by the photographic method. The Roberts-Nier method was reported in last year's review (*37*). Duckworth (*17–19*) and co-workers have a modified Dempster-type instrument in operation and have reported new measurements. Ogata (*62*) has measured the isotopic weights of some of the elements of medium weight. This general field has been an active one, with the Chicago group taking a leading role (*1*, *16*, *20*, *26*, *27*, *29–32*, *36*, *44*, *46*, *51*, *53*, *54*, *61*, *64*, *72*, *75*). Dempster and Shaw (*12*) have made an interesting observation on the relative retardation of high speed ions by the residual gas and have shown significant resulting differences in the pairings of doublets.

MASS SPECTRA

The American Petroleum Institute Research Project 44, conducted at the National Bureau of Standards, Washington, D. C., has accumulated 436 mass spectra of various gases and vapors, including paraffins, alkyl cyclopropanes, alkyl cyclobutanes, alkyl cyclopentanes, alkyl cyclohexanes, mono-olefins, diolefins, alkyl cyclo-olefins, acetylenes, diacetylenes, alkyl benzenes, and miscellaneous hydrocarbons. Various spectra have been reported: pentaborane by Dibeler, Mohler, Williamson, and Reese (*13*); hydrogen deuteride by Friedel and Sharkey (*22*); octanes by Bloom, Mohler, Lengel, and Wise (*6*); various organic compounds by Roberts and Johnson (*66*); hydrogen and deuterium by Bauer and Beach (*3*). Mohler, Bloom, Wells, Lengel, and Wise (*59*) discussed the doubly charged ion spectra in the mass spectra of hydrocarbons. Bloom, Mohler, Wise, and Wells (*7*) have compiled the metastable transition peaks in the mass spectra of about 170 hydrocarbons. The effect of temperature on mass spectra has been studied by Stevenson (*74*).

INSTRUMENTATION

Mariner and Bleakney (*56*) have described their large mass spectrometer employing crossed electric and magnetic fields. A resolution of 1 in 25,000 was expected from this instrument. Another such spectrometer is planned and in construction.

Bennett (*4*) has described a radio-frequency instrument which may be developed into a good analytical spectrometer. It is small, sensitive, and relatively inexpensive, and may some day be the poor man's mass spectrometer. Hipple, Sommer, and Thomas (*38*) reported a magnetic resonance method of ion analysis. Their instrument attained a resolution of 1 in 3500. The device employed no slits, and its high sensitivity and readily variable resolution suggest that it may be useful for gas analysis as well as precise mass measurements. It, too, is small and relatively inexpensive.

A mass spectrometer for continuous gas analysis has been developed by Hunter, Stacy, and Hitchcock (*41*). It is designed for respiratory studies, with three exit slits, for nitrogen, carbon dioxide, and oxygen. The time of response is fast, permitting determinations every 0.2 second.

Progress has been made in precision instruments for the measurement of packing fractions. Richards, Hays, and Goudsmit (*65*) constructed an instrument based on principles previously reported. An accuracy of 1 in 10^3 near mass 100 was attained.

An isotope ratio mass spectrometer of the Nier type has been made commercially by the Consolidated Engineering Corporation. Similar instruments are described by Paul (*63*) and by Lewis and Hayden (*52*). General analytical and isotope ratio mass spectrometers have now been made commercially in England by Metropolitan Vickers, and in France by the Compagnie Générâle de Télégraphie Sans Fils. A leak-detector mass spectrometer utilizing a cold cathode ion source has been developed by Thomas, Sommer, and Wall (*77*). Hipple and Thomas have reported a time-of-flight instrument (*39*).

A metal mass-spectrometer tube with guarded gold gasket has been described by Hickam (*33*). Both ends are conveniently removable, which is rather nice. Bernas and Nier (*5*) have described the production of an intense ion beam, and Winn and Nier (*79*) have developed an excellent emission regulator. Dibeler and Taylor (*15*) have described glass diaphragm leaks.

Theoretical studies of improved focusing have continued (*23, 35, 47, 48, 69, 70*). The production of ions has received some attention (*28, 45, 73*).

REVIEWS

Several reviews of the subject have been published, including those by Hutter (*42*), Inghram (*43*), Roth (*71*), Boivin (*8–10*), Thode (*75*), and Kistemaker (*49*).

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ELECTRON MICROSCOPY

F. A. HAMM, ***General Aniline and Film Corporation, Easton, Pa.***

ALTHOUGH there were no singularly outstanding contributions in techniques or in instrumentation during the past year, real progress has been made in electron microscopy as an applied science. Most critical investigators have stopped worrying about pushing resolution to the very ultimate—say 5 to 10 A. In general, the electron microscope is now being used for the chief purpose for which it was originally intended, the examination of structure improperly resolved in a light microscope. Certainly theory (*15, 17–19, 67*) and instrumentation (*9, 55, 56, 58, 61, 62, 64, 65, 73, 76*) are still under consideration; this is as it should be. However, the most gratifying development during the past year has been the reports of the applications of electron microscopy to important long-range research problems with subsequent more affirmative explanations offered for the solutions to these problems. There is much less concern about "artifacts." The workers are in general less skeptical and more confident. This greater feeling of security was expressed in the rather positive statements made throughout the papers (*23*) presented at the seventh annual Electron Microscope Society of America meeting (Washington, D. C., October 6, 7, and 8, 1949); these statements lend support to the feeling that electron microscopists are now gaining confidence in their approach. This development has not been hasty, nor has the pinnacle of success been reached. But as Burton (*11*) pointed out in his review of a year ago, electron microscopy as an analytical tool has proved to be of immeasurable value, and its future holds much promise.

Although the biological applications still command a great deal of attention, the workers engaged in metallurgical problems are now more willing to disclose their results. Those working with metals have been deliberate and cautious in their claims. Their fruitions have long been awaited. Their reliance on replicas is almost a necessity; the controversial aspects of the interpretation of the electron microscope image of this type of specimen justify their reluctance to commit themselves. The Symposium on Metallurgical Applications of the Electron Microscope (sponsored by the Electron Microscopy Group of the Institute of Physics, London, November 16, 1949) was the first of its kind. These metallurgical problems afford an excellent opportunity to use both the light microscope (metallograph) and the electron microscope in a corroborative way.

Refinements in specimen preparation techniques have played an important role in aiding the electron microscopist to gain confidence. However, a more significant reason may well be the realization that a few "snapshot" electron micrographs are wholly inadequate as a basis for explaining the complexities inherent in most problems. For example, the structure of bacterial nuclei, the mechanism of cell reproduction, the role of bacteriophage in lysis, etc., may be illustrated by a relatively few micrographs, but the critical investigator will base his conclusions on many micrographs and on observations of perhaps hundreds or thousands of different fields of the complex organic system. Now that such studies have been carried on for approximately a decade, more conclusive light is being shed on these problems. To put it another way, electron microscopists, especially those concerned with living complex biological structures, have accumulated a background of knowledge over a period of years, but only recently has it been possible in some cases to assemble a coherent picture. The electron microscope is no panacea. Although the next few years may bring results which may appear startling and command much publicity (especially in the biological field), this will only be the natural result of intelligent use of the instrument for purposes for which it was originally designed.

Perhaps the most significant development during the past year has been the application and refinements of the slow-speed microtomy technique, first described by Pease and Baker (*52*), in which a conventional rotary microtome was used. It would seem that, at least for the usual applications, the era of high-speed microtomy (*11*) has all but vanished.

INSTRUMENTS

There are now approximately 350 electron microscopes dispersed throughout the world. Of these some 330 instruments have been sold by the Radio Corporation of America, and most of them are located in this country.

It may be surprising to learn (*46*) that seven electromagnetic microscopes and two electrostatic instruments are made in foreign countries. It is believed that three different instruments are being manufactured in Japan, although there appears to be no literature available. Thus, with some twelve foreign instruments available, reliance on this country for equipment has been obviated.

Again there have been no outstanding developments in instrumentation in this country. In fact, a somewhat greater interest in this subject was demonstrated at the Conference on Electron Microscopy (*15*) held at Delft, Holland, July 4 to 8, 1949. The combination microscope–diffraction camera built for the Royal Dutch Shell Oil Company by the Philips Company is reported to be a noteworthy development. Although the magnification is fixed at 3000, the conversion from microscope to diffraction camera (and vice versa) by merely throwing a switch is a commendable contribution. The rapid exchange of specimens (pumping time 15 seconds) and the quick change-over to diffraction are notable features of the commercial Philips instruments (*23*). The 400-kv. Delft-Philips instrument is apparently working out successfully, although not much publicity has been attributed to the gain in penetration by the higher speed electrons.

An excellent discussion of the illuminating system of the RCA EMU electron microscope by Hillier and Ellis (*33*) describes the optimum conditions for using a self-biased gun. This paper is an aid to those who are interested in learning more about the functions of the various components in the microscope column. The removable intermediate lens described by Hillier (*23*) should prove valuable where a wide variety of magnifications is desirable. Grube (*20*) points out that stray magnetic fields may be internal, arising from local currents through the chassis or column. Dual grounds may be responsible if the instrument is grounded to a water line as well as through an electrical neutral.

Ramberg (*55*) in his mathematical treatments has shown how theoretically it might be possible to eliminate spherical aberrations in present-day objective lenses. He (*56*) has also explained by means of theory the phase contrast in the image of thin films. The inner potential of the film and its thickness become especially significant at small objective angular apertures. Borries (*9*) in Germany treats electron scattering and image formation in a general way. Scherzer (*65*) has very clearly discussed resolution as a function of focusing, illumination, aperture effects, and substrate thickness. His other papers (*66, 67*) are interesting from the standpoint of theory. Hall (*22*) has pointed out how anyone can determine the aberration constant for the objective lens employed by carefully measuring the displacement of the diffraction image (crystal edge) in a high-quality paraxial image. Thus, such an evaluation plus an evaluation of objective lens astigmatism (*23*) will rather accurately prescribe the quality of an objective lens. A magnetic beam-splitting focusing device described by Bishop (*8*) is similar in principle and effect to the older electrostatic deflection plate gadget. Gabor's (*17*) ingenious "diffraction microscopy" is still looked upon with intense interest. He has "synthesized" light microscopical images by reconstructing the diffracted wave fronts. His postulated analogous electron-optical reconstruction is intriguing, and is keenly awaited. The application of wave mechanics to electron imagery is capably treated by Glaser (*18, 19*). Electrostatic lenses from the standpoint of theory are discussed in several papers (*47, 49, 57, 58*). Theoretical discussions of the electron beam are given in several references (*62, 63*). Seeliger (*74*) discusses limitations in electron lenses due to apertures. Again in regard to lenses, Sixtus (*77*) in Germany discusses magnetic alloys of high permeability. Merling (*45*) describes a new fluorescent screen for the electron microscope.

APPLICATIONS

General Technical. The results of much electron microscopical research, especially in the industrial laboratories, is not disclosed because of the confidential nature of the problems. This is in some respects unfortunate. By comparison with the biological field, relatively little has been published on pigments, catalysts, fibers, photographic emulsions, and other similar technological products. During the early stages of development of electron microscopy as an applied science, many of the applications were more or less confined to routine particle size measurements. This is no longer true. Today the structure of particulate solids as related to their manufacture or use is being considered from the more academic point of view. The papers (*23*, Washington, D. C., Electron Microscope Society of America meeting) on grease, cellulose fibers, resinography, etc., substantiate this statement.

Watson (*81*) has illustrated the effects of aging on the structure and gel properties of vanadium pentoxide sols. The same author (*80*) has critically investigated various carbon blacks and has shown such structures as hollow spheres, ellipsoidal spheres, laminated spheres, and dumbbell pairs of spheres to be present. A novel and informative paper on alkali soaps by Hattiangdi and Swerdlow (*27*) serves to show how the structure and general morphology of alkali metal palmitates and stearates can be used for their characterization. Robillard (*60*) in France has used the electron microscope to study the application of antireflection coatings on optical glass. The structure of indium sulfide (optical and electrical properties) has been investigated by Schaffernicht and Helbig (*64*). Kossel (*38*) has been concerned with electron interferences in crystalline materials. Turkevich and Hillier (*79*) in a very general paper on colloidal systems of many types discuss how size distribution and morphology may be related to past history. A rather complete literature résumé is included.

Metallurgical. Heidenreich (*29*) has presented an excellent summary of his rather extensive study of metal crystal structure, using both the electron microscope and diffraction camera, and has given a dynamic theory of electron diffraction. He deserves much credit for developing an electrolytic polishing (thinning) technique for preparing thin metal sections. An interesting application of the relatively unknown shadow electron microscope is used to identify some of the lattice indexes. The bending, orientation, thickness, etc., of crystals of aluminum and copper-aluminum alloys in regard to structure are discussed. Schwartz *et al.* (*69*) utilize a two-step plastic replica technique for repeatedly replicating the same area. A positive Formvar replica is made from a negative water-soluble polyvinyl alcohol replica. Koch and Wiester (*36, 37*) in Germany have studied the distribution and role played by carbides in steels. Seeliger (*73*) in the same country has been concerned with the measurement of surface roughness of metals. The relation between metallic friction and surface damage under light loads is described by Whitehead (*82*). Robillard (*59*) has written a general review of the applications of electron microscopy to metallurgy.

In general, the year 1949 presented a preview of greater things to come from those engaged in metallurgical problems. A quantitative evaluation (*23*) of the percentage of carbide in heat-treated steels is a typical example of such studies. The effects of certain phases (graphite, carbides) on the physical properties of steels can be better evaluated if this approach is correlated with metallographic techniques.

Biological. Despite the fact that electron microscopists must be contented with their observations of "dead" organic systems. whose structure and activities during normal life are sought, tremendous progress is continuously being made. Obviously, there have been mistakes in interpretation. This is of little significance if the long-range aims are considered objectively. Investigators have profited through mistakes, and in the final analysis, the outstanding contributions in this field will have been strengthened by the perseverance of the investigators. The fruits of many years of labor are just beginning to be harvested. It is refreshing to read and hear conclusions being drawn with vigor and little reservation. A stimulating new book by Schmitt (*68*) should be read by all persons interested in biological applications of electron microscopy. The most voluminous results still come from the National Institutes of Health and the Massachusetts Institute of Technology under the direction of Wyckoff and Schmitt, respectively. The caliber of their work is such that these laboratories enjoy a pre-eminent place in the field. As in the case of metallurgical problems, the biological papers presented at the last Electron Microscope Society meeting suggest a new era in regard to both quality and quantity of published work.

Williams and Steere (*84*) in a study of tobacco mosaic virus have demonstrated how a very mild distilled water treatment causes compact bundles of the virus to disperse into individual rods. The need for considering the history of the specimen is immediately apparent if critical evaluations of morphology are to be made. By a similar token the effects of heat on the granulation of *E. coli* have been pointed out by Hedén and Wyckoff (*28*). The structure of bacteria nuclei, still a nemesis, has been partially elucidated by Hillier *et al.* (*34*). A network of large protein molecules has been resolved. Relatively little has been said, with much certainty, about the structure of the thin bacteria cell wall. On the other hand Knaysi and Hillier (*35*) have demonstrated in a positive way the mechanism of germination of *Bacillus megatherium*. The older theories of spore coat structure and disintegration have been refuted. The role played by bacteriophages and the mechanism of lysis are slowly but surely being clarified. In this regard a paper by Parmelee *et al.* (*51*) concerning bacteriophage active against *Streptococcus lactis* is significant. Wyckoff (*85*) in one of many studies has reported on the development of bacteriophage grown on solid media.

It is only natural that some of the older theories based on light microscopical observations are being proved to be incorrect. As an example of this, reference to a study of oral treponemes and *Treponema pallidum* by Hampp *et al.* (*24*) is cited. The general picture of filamentous and flagellar appendages and granular structure has been elucidated.

The structure of the nuclear membrane in bacteria, a difficult subject, has been investigated by Callan *et al.* (*12*). The interaction of viruses with fowl red cell membranes has been studied by Dawson and Elford (*16*). Similarly, a study of the interaction of human red cells and the influenza virus has been reported by Heinmets (*31*). The virus of tobacco leaf curl has been examined by Sharp and Wolf (*75*).

An early paper on a portion of a most interesting and extensive investigation of human tooth structure, by Scott *et al.* (*70*), describes the effect of age on tooth surfaces. Similar papers resulting from this long-range program at the National Institutes of Health will appear in the future. Lindemann (*40–42*) in Germany has studied the blood system with particular attention to the erythrocytes. Mühlethaler (*48*) has worked with plant fibers, a relatively unexplored field. Zahn (*86*) has studied natural silk. The electron microscopy of F-Actin has been reported by Rozsa *et al.* (*61*). Fibrinogen and fibrin, of long interest to the

Massachusetts Institute of Technology group, have been reported on by Hall (*21*). A recent application of ultrasonic waves to bacteria and viruses has been described by Hamre (*25*).

One of the more startling and certainly a fascinating report was made by Pease and Baker (*53*) concerning their resolution of chromosomes and genes. This report was meant to be only preliminary and the results require verification. The detection of these important bodies was facilitated by their thin-sectioning technique (*52*). Biological specimens in general, the subject of a British conference at Cambridge, have been reported on by Cosslett (*14*).

Because the two-component objective described by Hillier (*32*) is now several years old, and because it affords a high gain in image contrast, it is somewhat surprising that this lens has not been more extensively used for studying the internal structure of biological systems. Admittedly, this lens introduces spherical aberrations and the resolving power may be relatively poor (75 A.); still the tremendous gain in contrast in images which are normally virtually void of contrast (in the absence of shadowing) would seem to outweigh the limitations for many applications.

There are strong differences of opinion concerning the preparation of specimens of bacteria, viruses, etc. The effects of prolonged washing, acid fixation, drastic centrifugation, etc., after broth culture are real, and proper cognizance must be taken. Growing the organisms on the substrate which rests on the solid nutrient agar appears to have certain advantages. However, this subject is open to strong argumentation from all sides. Perhaps this is a healthy atmosphere, because the whole subject of electron microscopy hinges on the correct consideration of the specimen history. Brieger and Cosslett (*10*) have considered this controversy and have reported their technique for preparing bacteria specimens with a minimum of disturbing interferences. Centrifugation is avoided.

SPECIMEN PREPARATION

The technique for properly preparing an electron microscope specimen often requires the skill of an artist as well as a scientist. Great strides have been made, and this is one reason why electron microscopists are more willing to commit themselves. Although those concerned with biological specimens have their troubles, those confronted with metallurgical or similar solid, electron-opaque materials have been even less fortunate. Much credit is due to Williams and Wyckoff for developing the now well established techniques for replicating and shadowing a variety of specimens. It is highly probable that techniques designed for special materials have never been published. This may be the consequence of the highly competitive nature of the particular industrial material.

Williams and Backus (*83*) in a highly informative paper summarize a variety of detailed procedures for shadow-casting and replica reproduction, and discuss the effect of "substrate grain" on the resolution of biological specimens. The two-step positive plastic replica technique as applied to metals by Schwartz *et al.* (*69*) has been discussed above. Heinmets (*30*) has employed rotary condensation of silica and metal vapor to biological systems. The deposition of vapor as the specimen slowly rotates through 360° is reported to permit greater resolution in certain areas because the shadow effects are less harsh. Hass and Kehler (*26*) very adequately describe their procedures for the examination and preparation of replicas and films of vaporized silicon monoxide.

Singer and Petzold (*76*) report the casting of a 1% ethylene dichloride solution of ethylcellulose onto preshadowed specimens. Tearing is apparently minimized and stripping of the replica is facilitated. Comer and Hamm (*13*) report the use of a variety of sources of silicon dioxide with a comparison of vaporizing conditions. The specifications for metal shadowing required by certain biological tissues are described by Scott and Wyckoff (*71*). Trotter (*78*) shows evidence to support his statement that the upper (air) surfaces of cast collodion replicas are not flat. Shadowing of these one-step replicas must therefore be done on the contact side. The production of silica films (replicas of Formvar) in large quantities is described by Bishop (*7*), who (*6*) has designed a clever device for automatically controlling the thickness of evaporated metals (shadowing). The heating filament is shut off when the condensed metal layer attains a certain thickness. This is done electronically; calibration is necessary. Bishop (*5*) has also devised a scheme for producing a readily identifiable area on the specimen screen. Like many others, his scheme is based on a hole punched in the middle of the wire mesh. Barski *et al.* (*4*) in a French publication describe a method for mounting disintegrated cellulose fibers. The novel and potentially useful technique of Heidenreich (*29*) for electrolytically thinning metals so as to be examined per se in the electron microscope deserves mention again.

No discussion of specimen preparation would be complete without reference to the Dow polystyrene latex spheres 580-G. Although the subject of some skepticism and controversy, it has now been well established (*23*) that these spherical particles are extremely useful as an internal calibration standard. Backus and Williams (*1*) in a critical study have pointed out three significant uses of these spheres: (1) magnification calibration, (2) as an aid in interpreting contour surfaces (replicas), and (3) as an aid in calculating the thickness of the shadowing metal. Until something better comes along, these uniformly sized polystyrene spheres will be put to good use. Scott (*72*) has reported certain irregularities in this same Dow latex sample: lack of sphericity and size uniformity. He recommends standardization of mounting techniques. It may be that Scott's findings are the result of the effects of the electron beam.

The wealth of new information pertaining to slow-speed conventional microtomy presented at the Washington Electron Microscope Society meeting (*23*) substantiates an earlier statement to the effect that high-speed microtomy (up to 50,000 r.p.m.) is essentially a thing of the past. Baker and Pease (*2, 3, 52*) in a series of papers have demonstrated the advantages of using a hand-operated conventional rotary microtome (Spencer Model 820). Obviously refinements are possible and will be reported. The mounting and fixation technique is often lengthy and must be done critically, but the preparation of high quality sections of bacterial (*B. megatherium*) cells and a variety of body tissue cells, some 0.1μ (or less) thick, is indeed fascinating. Uniform thickness with a minimum of distortion are two factors that add weight to the significance of this technique. Laurell (*39*) reports a sectioning of bacteria in situ by stripping a thin layer of evaporated beryllium from the specimen. Some of the cytoplasmic layer is retained. The general applicability of this technique appears doubtful. Newman *et al.* (*50*) have successfully modified the technique described by Baker and Pease for slow speed conventional microtomy. The novel features of their procedure are specimen advance during sectioning by the thermal expansion of the supporting brass block, and embedment of the specimen in a polymer which had formed around the specimen from the liquid monomer.

ELECTRON DIFFRACTION

Electron diffraction is a relatively old science and is a vast subject in itself. Its close relationship with electron microscopy justifies its consideration in this review. Strangely enough, there has been a dearth of electron diffraction studies reported in the literature. This is surprising and at the same time disappointing. The very fact that many electron microscope specimens lend themselves admirably to diffraction is just cause for surprise. Furthermore, the two RCA universal microscopes (Types EMB and EMU) and several foreign instruments are readily adaptable to diffraction. Perhaps the more widely applicable and more common x-ray diffraction technique is used instead. However, it is hoped that wherever practical (limited sample available or thin film specimens) this auxiliary electron diffraction technique is not being overlooked. The transfer of thin surface structure (dyestuffs, alloy inclusions, surface films such as oxides, etc.) to a replica or by means of a similar stripping process, deserves the attention of many electron microscopists.

Mahl (*43*, *44*) in Germany has reported on the subject in a general way. A particularly informative paper has been written by Picard *et al.* (*54*). The new RCA electron diffraction unit is described and its universal features are discussed. However, the significant aspect of this paper is the valuable information concerning the optics, resolution, accuracy, and application of the diffraction unit. This critical discussion of the lens system and its relation to resolution and accuracy deserves to be read by all concerned. Applications of this well designed instrument should contribute much to our knowledge of structure and identification.

The instruments built by the Philips Company, discussed above, should also stimulate the use of electron diffraction as an analytical technique.

FOREIGN DEVELOPMENTS

It is only natural, now that the disturbing influences of the war have abated, that foreign electron microscope societies should be formed. There are four well organized societies abroad, representing England, Germany, The Netherlands, and Sweden. Meetings are being sponsored by these societies, and it is likely that more international meetings will be held within the next few years.

Cooperation between the Electron Microscope Society of America and the foreign groups is already in effect. To this end, the executive council of the Electron Microscope Society, representing its entire membership, has sent as a gift a complete set of keysort bibliography references to the secretaries of the four active foreign societies. The dissemination of knowledge and mutual assistance wherever possible are fostered by all concerned. V. E. Cosslett in England is preparing a bibliography of interest to electron microscopists, as P. C. Smith has done for the Electron Microscope Society of America.

Because this information is not readily available, it seems appropriate at this time to include the names and addresses of the secretaries of these foreign societies.

V. E. Cosslett, Secretary, British Electron Microscopy Group,
Cavendish Laboratory, Free School Lane, Cambridge, England
Habil Bodo V. Borries, Secretary, Deutsche Gesellschaft für Elektron Mikroskopie, Direktor des Rheinisch-Weltfalischen Instituts für
Ubermikroskopie, August Thyssen Strasse, Dusseldorf, Germany
W. A. Le Rutte, Technische Physische Dienst T.N.O. En. T.H.,
Labrotorium voor Technische Physica der Technische Hoogeschool,
Mijnbouwplein 11, Postrekening No. 452194, Delft, Netherlands
Fritiof S. Sjostrand, Secretary, Swedish Electron Microscopy
Group, Karolinska Institutet, Anatomiska Institutionen, Solmavagenl, Stockholm, Sweden

Meetings. Undoubtedly there have been lesser known meetings, but two meetings held abroad deserve mention at this time. The Applied Physics Section of the Netherlands Physical Society organized a conference on electron microscopy at Delft July 4 to 8, 1949, which was attended by workers from many countries. Instrument demonstrations as well as theoretical and applied papers served to show the rapid progress made in Europe since World War II. This meeting has been reviewed by Cosslett (*15*).

The second meeting, not reviewed at the time of this writing, was held at London, November 15 and 16, 1949, sponsored by the Electron Microscopy Group of the Institute of Physics. This meeting was highlighted by a Symposium on Metallurgical Applications of the Electron Microscope. Virtually every technical society in England was represented by the authors of the many papers.

New Journals. Because of the wealth of research that has been carried out abroad, much of which has not been accessible in published form, it seems appropriate to mention new journals which will include papers of interest to electron microscopists: *Microscopie*, Editions de la Revue d'Optique Théorique et Instrumentale, Paris, France; *Optik*, Wissenschaftliche Verlagsgesellschaft m.b.H., Stuttgart, Germany; and *Physikalische Blätter*, Verlag Volk und Zeit, Karlsruhe, Germany. The workers in this country may do themselves a service by acquainting themselves with the developments abroad.

FUTURE DEVELOPMENTS

This is no attempt to predict the future. Nevertheless certain trends are appearing, and it is only logical that they be considered. The utilization of higher speed electrons in the 100- to 200-kv. range for greater penetrating power seems certain. Close scrutiny of this suggests that in some specimens the overlapping of structure may preclude the possibility of gaining much more information. Still higher voltages may be used for special applications, but power supply stability may be a restraining factor. Although the x-ray microscope is an interesting experiment, and it would furnish the necessary penetration, it is still much too early to expect any significant contributions from this instrument. Likewise, the proton microscope has serious limitations and may find only very limited application. Damage imparted to the specimen, power supply stability at the necessary high voltages, and the usual difficulties with electrostatic lenses do not add up to a bright future for the proton microscope.

It is gratifying that the use of the electron microscope at low magnifications is now becoming more routine. Time and effort have been wasted in the past in worrying about the optimum resolution at the maximum instrument magnification. More common sense is now evident in the application of the instrument to problems which also lend themselves to other instruments such as the light microscope and metallograph. One point stands out in bold relief—the electron microscope should never be used at magnifications higher than absolutely necessary.

Finally, the electron microscope, despite all the difficulties associated with it and its techniques, has established itself and is more than holding its own beside the many other instruments in modern research laboratories. Knowing this, and adding it to the greater feeling of confidence enjoyed by the workers in the field, points to 1950 as the year during which a record number of high quality reports will be forthcoming.

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ORGANIC POLAROGRAPHY

STANLEY WAWZONEK
State University of Iowa, Iowa City, Iowa

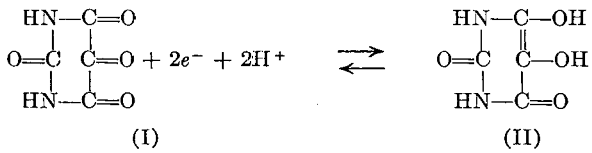
IN THE past year the field of organic polarography has seen considerable work done on the reduction of nitro compounds, repetition of previous work, and further application of the polarographic method to the quantitative determination of organic and inorganic compounds.

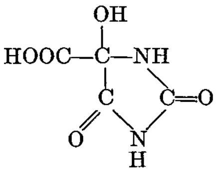
Most of the investigations have been carried out in mixtures of water and various organic solvents. For difficultly soluble compounds such as oxidized fat and gasoline peroxides, a mixture of equal volumes of benzene and methanol has proved useful (*21*).

The use of glacial acetic acid as a solvent is suitable only up to a voltage of −1.1 volts. The Ilkovič equation can be applied in this medium with satisfactory accuracy (*11*).

REVERSIBLE SYSTEMS

The data reported on alloxan(I) and dialuric acid(II) indicate that this system is reversible. Alloxan(I) gives two reduction waves at +0.182 and −0.588 volt, whereas dialuric acid(II) gives an oxidation wave at +0.182 volt. The more positive wave is for the reversible system, while the wave at −0.588 volt is probably a catalytic hydrogen wave brought on by the —NH—CONHCO group, since a similar value is given by alloxanic acid (III). Alloxantin gives an oxidation wave at +0.182 and a reduction wave at −0.588 volt (*43*). Mesoxalic acid and tartronic acid, the open-chain analogs of alloxan and dialuric acid, show no oxidation or reduction waves (*42*).


(I) (II)


(III)

The work with other reversible systems has been mainly concerned with their quantitative determinations.

Pyrogallic acid and pyrocatechol give anodic waves which can be used for their estimation. Resorcinol and phloroglucinol are not oxidized under the same conditions. This method has been used to study aqueous quebracho extracts (*55*).

The decomposition of lactoflavin (vitamin B_2) by light has been followed polarographically in 30% *N*-methylacetamide solution, and lumichrome and formaldehyde have been identified as decomposition products (*2*).

Substituted azobenzenes such as methyl red (*35*) and methyl orange (*34*) give waves suitable for their determination. The wave of the latter is decreased 10% by 0.01% gelatin. Larger amounts of gelatin up to 1% have no further effect. Serum albumin reduces the wave by combination with the methyl orange until the protein concentration is 0.5% for 10^{-4} molar methyl orange. From the diffusion coefficient of the complex and the decrease of i_d at various concentrations of the protein, the molecular weight and the binding constant, respectively, can be calculated. No effect of the serum albumin on the diffusion current is noticed in alkali (*49*).

IRREVERSIBLE SYSTEMS

Aldehydes. The effect of hydration upon formaldehyde has been studied further, and the heat of hydration (13,000 calories per mole) calculated from the temperature-diffusion current curve. The formaldehyde wave can be removed in 16 minutes by the addition of dimedon (*26*).

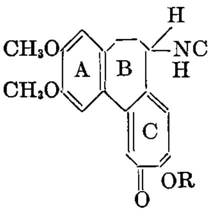
The polarographic method has been used to determine formaldehyde in commercial preparations (*37*); it can be used indirectly to determine glycerol in fermentation residues by oxidizing the latter to formaldehyde with periodic acid and then distilling (*7*). This method is similar to that previously reported for ethylene glycol and 1,2-propylene glycol (*56*). In the latter case it is possible to determine the amounts of diglycols (diethylene and dipropylene) by removing the monoglycols with periodic acid oxidation and distillation and then oxidizing the residue with potassium dichromate. The dichromate is measured polarographically before and after the oxidation (*9*).

Fructose, which shows a behavior similar to formaldehyde due to hemiacetal formation, was studied in solutions of various inorganic salts and found to give the best wave with calcium or lithium compounds at a pH of 7.0. The diffusion current in the former is 1.6 times as high as that in the lithium salt solution (*54*).

Unsaturated aldehydes in which the aldehyde group is conjugated with a double bond are best determined at pH 5 to 6, because under these conditions peroxides, if present, do not interfere with the early wave shown by these compounds (*26*).

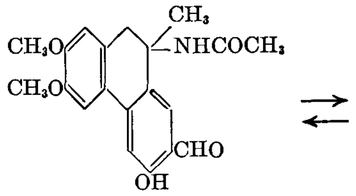
Aromatic aldehydes to a large extent show similar polarographic behaviors. Benzaldehyde, for example, shows two waves between pH 2.6 and 6.0 which merge into one wave at a pH greater than 6.2 (*17*). *p*-Dimethylaminobenzaldehyde, protocatechualdehyde, *p*-nitrobenzaldehyde, chloronitrobenzaldehyde (*38*), salicylaldehyde, anisaldehyde (*1*), *p*-hydroxybenzaldehyde, and *p*-methoxybenzaldehyde (*41*) behave similarly and differ only in the pH at which the two waves merge into one.

The behavior of salicylaldehyde and *o*-anisaldehyde has been compared with that of colchicine (IV) and colchiceine (V) in an effort to gain information about the structure of ring C.

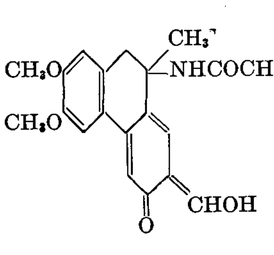


(IV)

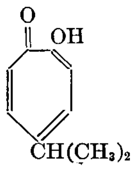
IV R = CH_3
V R = H



(V)



(VI)



(VII)

Both colchicine(IV) and colchiceine(V) give a two-stage reduction in acid solution of one electron each and a single two-electron step in alkaline solution. The behavior of colchiceine (V) actually parallels that of a compound having acid dissociation. It combines with boric acid to give a complex which gives only one wave and is not stable in alkali (*1*). A similar behavior is observed with thujaplicin(VII) and is good evidence for a seven-membered ring (*1*). The possibility of a hydroxymethylene structure(VI) was eliminated by the fact that compounds having this structure—2-hydroxymethylene-1-ketotetrahydrophenanthrene, 3-hydroxymethylene-4-ketotetrahydrophenanthrene (*41*), and hydroxymethylenecyclohexanone—gave a more complicated behavior and showed three reduction waves.

The polarographic method has been used to detect and determine benzaldehyde and cinnamaldehyde in pharmaceutical preparations (*38*).

The behavior of furfural parallels that of benzaldehyde at the dropping mercury electrode. Quantitative estimation can be carried out in sodium acetate solution with good results even in the presence of formaldehyde (*24*). The method has been used to determine furfural in alcohol extracts of resins (*24*) and in the products formed in the deterioration of apricots (*22*). In the latter case furfural did not account for all the carbonyl compounds present. 5-Hydroxymethylfurfural, pyruvic acid, dihydroxyacetone, glyoxal, methylglyoxal, and acrolein were considered as possible products and were studied polarographically.

Ketones. The aliphatic ketones, acetone and methyl ethyl ketone, are reduced polarographically at −2.20 and −2.25 volts in tetramethylammonium iodide solution and can be determined with an accuracy of 5%. Formaldehyde, acetaldehyde, and citral do not interfere. No curves are obtained in acid, sodium hydroxide, or lithium chloride solution (*27*). These ketones can also be determined indirectly by determining the diminution produced in the sulfur dioxide curve of a sulfuric acid solution of sodium sulfite. The drop in current is a function of concentration but is not a linear one (*39*).

In the aromatic series the effect of one and two hydroxyl groups upon the ease of reduction of acetophenone has been studied and found to be the greatest (most difficult to reduce) when a hydroxyl group is in the *p*- position (*52*).

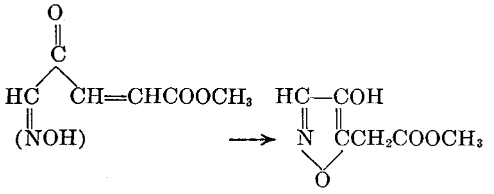
Half-wave potentials are reported for acetophenone, methylacetophenone, benzophenone, benzoylacetone, chloroacetophenone (*40*), and benzanthrone (*51*). The last compound can be determined quantitatively alone or in mixtures with anthraquinone.

The polarographic method has been used to follow the formation of chalcones from *o*-hydroxyacetophenones and aromatic aldehydes and the cyclization of these unsaturated compounds into flavanones (*44*).

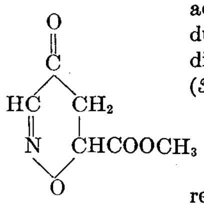
Acids. The polarographic method has been used in various ways with organic acids.

The nature of the reduction of fumaric and maleic acids and the oxidation of ascorbic acid has been studied using the oscillographic method and found to be irreversible (*13*). The half-wave potentials obtained for the oxidation of ascorbic acid are more positive than the potentiometric values by about 210 mv. It has been suggested that at the mercury drop, the product formed isomerizes to an irreversible inactive form (*53*).

The polarographic method has been used to study the rate of formation of the oxime and hydrazone of mesoxalic acid (*42*), the polymerization of methyl methacrylate in the presence of acetone peroxide, diethyl peroxide, and benzoyl peroxide (*28*), and the rate of decomposition of methyl 4,5-diketo-2-pentenoate-5-oxime(VIII) (*12*). In order to aid in interpreting the data in the last case, methyl 4-keto-2-pentenoate and 3,5-diphenyl-4-isoxazolol were studied. On the basis of the similarity of the polarographic data of the decomposition product to that of 3,5 diphenyl-4-isoxazolol it was suggested that an isoxazol(IX) is formed.



(VIII) (IX)

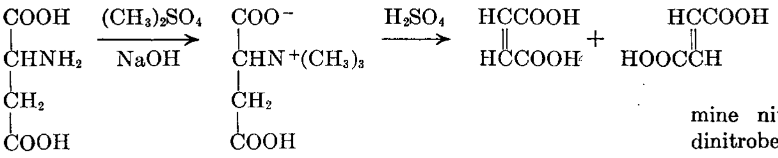


(X)

The possibility of an addition to the α,β-unsaturated ketone system and the subsequent formation of an oxazoline (X) was not considered.

The study of the behavior of the γ-aldehydo acid, phthalaldehydic acid, at the dropping mercury electrode has given results which have proved helpful in assigning definite structures to the anhydrides of the previously studied *o*-benzoylbenzoic acid (*58*).

Aspartic acid in acid-hydrolyzed proteins can be determined polarographically by conversion to a mixture of fumaric and maleic acids which gives a well defined wave (*57*).



$$\begin{array}{l}COOH\\|\\CHNH_2\\|\\CH_2\\|\\COOH\end{array}\xrightarrow[NaOH]{(CH_3)_2SO_4}\begin{array}{l}COO^-\\|\\CHN^+(CH_3)_3\\|\\CH_2\\|\\COOH\end{array}\xrightarrow{H_2SO_4}\begin{array}{l}HCCOOH\\\|\\HCCOOH\end{array}+\begin{array}{r}HCCOOH\\\|\\HOOCCH\end{array}$$

Organic Halides. The isomers of hexachlorocyclohexane in 80% ethanol with 0.1 N tetraethylammonium iodide as the electrolyte give half-waves at -2.02 volts (α), -2.15 volts (β), -1.61 volts, and -2.58 volts (γ). Impure (80%) δ-isomer gave a wave at -2.08 volts. The first wave can be used for determining the γ- isomer quantitatively (*45*).

The γ- isomer is the only one that is reduced in a potassium chloride–sodium acetate buffer and gives a long drawn out wave. Heptachlorocyclohexane interferes by raising the residual current but can be corrected for. The values obtained by the polarographic method are lower than those obtained by means of bioassay. The latter may be higher because of a synergistic effect of the heptachlorocyclohexane (*5*).

Nitro Compounds. The polarographic method has been used to study the rate of transformation by alkali of nitromethane, nitroethane, 1-nitropropane, and 2-nitropropane into the sodium salt of the *aci* form (*25*).

In the aromatic series considerable work has been done on the reduction of nitro compounds.

Nitrobenzene gives two waves of four and two electrons, respectively, at a pH less than 4 (*15, 31*). The second wave is not well defined and disappears at pH values greater than 4. The first wave represents a reduction of nitrobenzene to phenylhydroxylamine, while the second wave in acid solution represents the reduction of the salt of the latter to aniline.

The addition of alcohol shifts the waves to more negative potentials and brings out the second wave more clearly (*29*).

Substituted nitrobenzenes show varying behaviors depending upon the substituents present. Nitrotoluene (*31*), nitroanisoles, and methyl nitrobenzoates (*29*) differ in their behavior from that of nitrobenzene only in the pH at which the second wave disappears. Dinitrobenzenes and dinitrotoluenes show twelve electron reductions in acid media and ten or less in alkaline buffers (*31*). Trinitrotoluene and trinitrobenzene give currents corresponding to a reduction greater than twelve electrons in acid and a twelve-electron reduction in alkali (*31*). The nitrophenols resemble nitrobenzene in their behavior in acid solutions but differ in the case of the ortho and para isomers in that the four-electron wave increases to one of six electrons with an increase in pH. The dinitrophenols resemble the dinitrobenzenes. Picric acid shows a seventeen-electron reduction and is probably reduced to a hydrazobenzene which rearranges in acid to the benzidine. This product would be stable toward further reduction (*32*). Nitroresorcinols and dinitroresorcinols give six and twelve electron waves, respectively, at all pH's, while styphnic acid behaves like picric acid and gives a seventeen-electron wave (*33*). The nitrobenzoic acids give two reduction waves in both acid and alkaline buffers (*29*).

The substituents make the reduction of nitrobenzene more difficult in the following order: nitro > carboxyl > chloro > methyl. Nitro and carboxyl show a greater effect in the ortho and para position than in the meta, whereas chlorine and methyl which increase the electron density at the ortho and para positions make the meta more easily reduced (*3*). The values reported by these investigators (*3*) differ from those reported by Pearson (*31*) by 0.25 to 0.35 volt. This discrepancy is ascribed to a difference in the nature of the buffers.

Two methyl groups as in 2-nitro-1,4-dimethylbenzene and 3-nitro-1,2-dimethylbenzene have little effect upon the ease of reduction of the nitro group. Methyl groups substituted in a manner which inhibits sterically the resonance of the nitro group, as in nitromesitylene and nitrodurene, make the nitro group more difficult to reduce in acid and basic media (*8*).

The polarographic method can be used to determine nitrobenzene in the presence of aniline, benzidine, or dinitrobenzene (*16*) and 1-nitronaphthalene in the presence of 1-naphthylamine (*50*).

Benzene can be determined polarographically by conversion to dinitrobenzene using mixed acids (*46*). In the presence of its homologs, toluene and xylene, the nitrated products are oxidized and the acids removed with alkali before the polarographic analysis (*18*) is carried out.

Unsaturated Hydrocarbons. Cyclooctatetraene gives in the presence of tetramethylammonium ions a well defined wave at -1.5 volts (S.C.E.) which is independent of pH and involves a two-electron change. Because in other salt solutions the wave is indefinite, it has been suggested that the reduction proceeds in the following manner (*6*):

$$C_8H_8 + 2e^- + 2(CH_3)_4N^+ \longrightarrow [(CH_3)_4N^+]_2\, C_8H_8^{--}$$

Peroxides. Benzoyl peroxide, lard peroxide, methyl linoleate peroxide, gasoline peroxide, and ethyl, isopropyl, and *n*-butyl ether peroxides have been studied in a mixture of ethanol-benzene containing methyl hydrogen sulfate, lithium chloride, or lithium methoxide. Lithium chloride is the best electrolyte to use, because no hydrogen wave is formed nor is any peroxide destroyed (*21*). This method was used to follow the changes in lard (*20*).

Sulfur Compounds. Ethyl and *n*-butyl mercaptan give two anodic waves in ethanol as a solvent. The second wave is not reproducible (*10*).

In the cobalt-catalyzed reaction of cystine, straight lines result if the reciprocal of the wave height is plotted against either the reciprocal of the cystine concentration (at constant cobalt concentration) or the reciprocal of the cobalt concentration (at constant cystine concentration) (*14*).

The protein index of Muller and Davis is different for normal and pathological serums of horses, sheep, and rabbits (*36*).

Heterocyclic Compounds. The reduction potentials for a series of aldehydes and acids in the pyrrole series are reported (*4*).

The polarographic method is suitable for determining folic acid alone or in the presence of tablet constituents (*23*).

8-Hydroxyquinoline has been studied in buffers ranging from pH 2 to 12 and gives three waves. The first wave changes in height with pH and is probably a catalytic wave. The second is an absorption wave, while the third is a probable reduction of the quinoline molecule (*47*). This behavior of 8-hydroxyquinoline has been made the basis of a method for determining aluminum. The aluminum is precipitated with 8-hydroxyquinoline and the excess reagent used is determined polarographically (*30*).

Quinoline-8-carboxylic acid has been studied in buffers ranging from pH 1 to 12 and behaves like 8-hydroxyquinoline in that three waves are obtained (*48*).

Determination of Metals. The addition of 2-naphthol, thymol, and diphenylamine changes the diffusion current and shifts the half-wave potentials of metallic ions. This behavior is ascribed to adsorbed films upon the electrode (*19*).

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POTENTIOMETRIC TITRATIONS

N. HOWELL FURMAN, *Princeton University, Princeton, N. J.*

THIS review covers the major advances in the field of potentiometric titrations subsequent to a review published in 1942 (*73*). Much progress has been made in this period in the direction of more convenient apparatus, and both automatic and recording titration assemblies have reached the stage of commercial availability. Much attention has been given to titrations of various sorts in nonaqueous media. The major share of the papers have dealt with well established principles applied to new situations. If classified by countries in which they were published, the distribution of effort was: United States of America 24.3%, Russia 22.6%, Germany 12.8%, France 12.4%, and England 10.7%. The remaining 17.2% of the papers appeared in Argentina, Brazil, Canada, Czechoslovakia, Finland, India, Italy, The Netherlands, New South Wales, Poland, Spain, Sweden, and Switzerland.

GENERAL PRINCIPLES

The theoretical question of the offset between the inflection of a titration graph and the theoretical equivalence point has been re-examined mathematically by Murgulescu and Dragulescu (*139, 140*). They found a slight offset between these two points in unsymmetrical types of titrations.

The general theory and the applications of the potentiometric and related methods have been dealt with in a number of reviews by Carter (*32*), Fernando and Gumersindo (*65*), and Flatt (*66*). Special summaries have been published on the dead-stop end point by Böttger and Forche (*18*), on polarization end points by Evans (*63*), and on pH electrodes by Lauchlan (*112*).

APPARATUS

Electrodes. Perley (*151–153*) has made important detailed studies of glass electrodes of special compositions. Glasses of good stability and low sodium ion errors contained 63 to 67 mole % of silica, 1 to 4 mole % each of lanthanum, calcium and barium oxides, 24 to 26 mole % of lithia, and 2 mole % of cesium oxide.

Lykken and Tuemmler (*126*) have stressed the suitability of the glass electrode as a reference half-cell in processes of types other than neutralization. A convenient rotating electrode has been described by Brüggemann (*23*).

Hume and Harris (*96*) devised a simple bottle type of electrode with agar-filled salt bridge that has a low resistance, about one tenth that of a similar electrode with sintered-glass barrier. The electrode is finding much use in amperometric titrations.

Lykken and Rolfson (*125*) devised an improved titration stand that utilizes commercially available electrodes.

Vacuum Tube Voltmeters. Penther and associates (*150*) developed a vacuum tube voltmeter for use with electrodes of high resistance. Subsequently (*149*) this basic instrument was incorporated in a dual titration instrument with provision for two sets of electrodes. Titrations of the same or different types may proceed simultaneously with intermittent voltage readings. Buras and Reid (*24*) developed a line-operated titrimeter that is suitable for titrations. Lineweg (*114*) described a direct current instrument for use with electrodes of high resistance. Many alternating current line-operated pH meters and titration assemblies are now commercially available. This postwar development has been very rapid, and it has made the potentiometric method much more attractive for control, research, and instruction—for example, instruments of the Central Scientific Company, Macbeth Corporation, National Technical Laboratories (Beckman), Photovolt Corporation, Precision Scientific Co., etc. The use of simple magnetic stirrers has greatly improved the assemblies, particularly when titrations must be made with an inert atmosphere above the solution.

Most of the simplified methods of titration continue to be used, particularly the dead-stop method of Foulk and Bawden which has been reviewed (*18*). Galvanometric indication—i.e., observation of changes of current flowing from the titration cell through a high resistance and a galvanometer—has again been found useful by Chirkov (*38*).

Delahay's Methods. Delahay (*48–50*) has devised circuits for both potentiometric and conductometric titrations. The reagent is added at a constant rate, and the current, flowing through the cell, a condenser, and a sensitive meter, varies with time in a manner given approximately by: $i = C\,dE/dt$, where i is current, C is the capacity of the condenser, E is the e.m.f., and t is time. In potentiometric titrations there is a maximum value of i close to but beyond the equivalence point (*48*). Either indicating or recording assemblies may be based on this principle.

Automatic and Recording Instruments. A rapid development has occurred along lines of application of electronic devices to recording titration data or to the devising of mechanized titrimeters. Pompeo and associates (*159*) have developed mechanized assemblies. Müller and Lingane (*137*) adapted the Schmidt trigger circuit to the goal of stopping a titration as soon as the indicating electrodes reach a predetermined value. If this value is exceeded by 2 to 3 millivolts, a relay is activated to control a buret, preferably of the motor-driven syringe type. If the electrodes show a premature end point due to a local excess of reagent, the motor is activated by an impulse to a second relay and the titration is continued to a permanent change in e.m.f.

Robinson (*174*) adopted the principle of adding reagent by a motor-driven syringe buret operated in synchronism with the drive of a recording potentiometer. The latter is so modified that reagent is added rapidly when the potential is changing slowly at the indicator electrode; slow increments of reagent are made as the end point is approached. A limit switch stops the action at any desired point beyond the equivalence-point potential. Lingane (*115*) devised a similar automatic titration apparatus based upon the motor-driven syringe buret and a potentiometer recorder equipped with a switch to stop the action at any predetermined potential. The apparatus was shown to be well adapted to titrations of various types and was utilized in titrations with chromous solutions (*116, 120*). A very high degree of precision was attained with the instrument. Lingane (*117*) also devised a multipurpose electroanalytical servoinstrument that may be utilized for a large number of analytical goals, including automatic potentiometric titrations.

A plot of potential at the indicator electrode against time during which reagent is added at a constant rate has been used by Kale (*103*). Barredo and Taylor (*6*) applied this idea to automatic potentiometric titrations by amplifying the output of the titration cell and registering the potential against time on a recorder. Constant flow of reagent was attained with the aid of a Mariotte flask as a constant pressure head. A galvanometer recording device such as that described by Lykken *et al.* (*123*) for polarography should be of interest in registering current-time

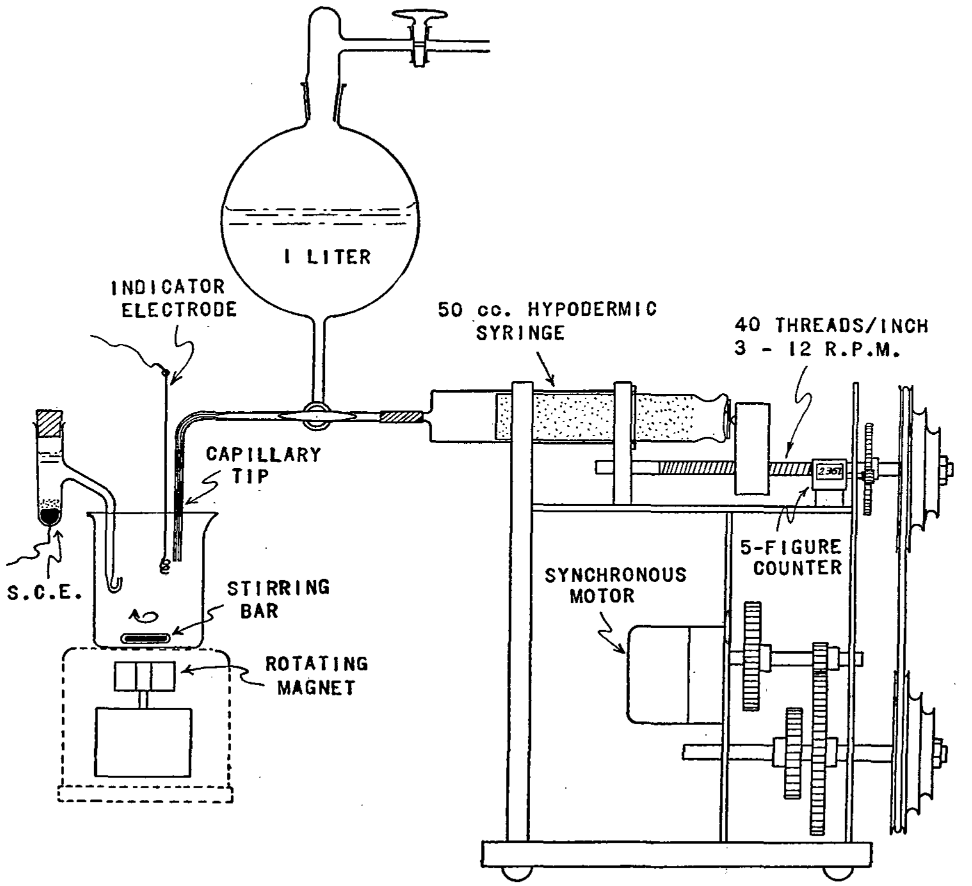
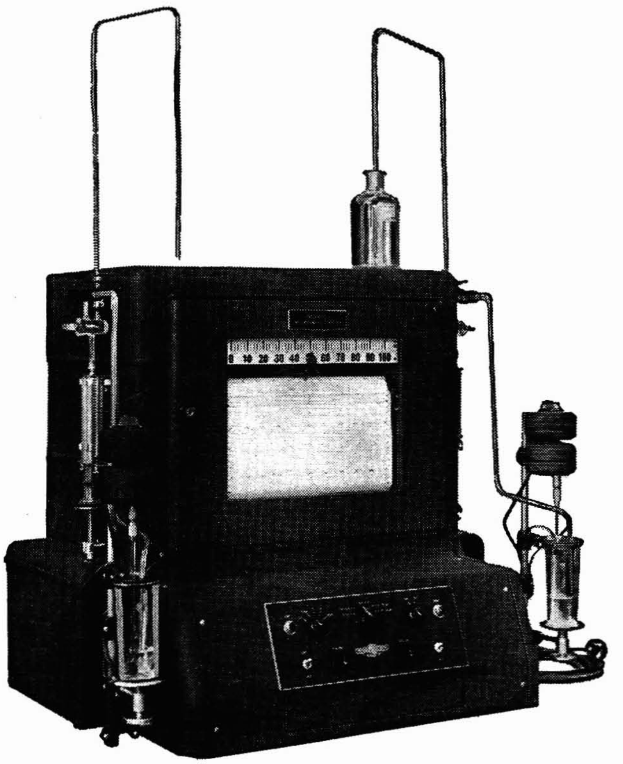


Figure 1. Autotitrator (*115*)



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Dual Recordomatic Titrator

plots and in coulometric titrations. Other automatic devices have been described by Portnov (*160*) and by Guntz (*91, 92*).

Coulometric Titration with Potentiometric Indication. The determination of a substance by oxidation, reduction, or other reaction at a "generator" electrode by measuring the time at which a constant current has brought a specific reaction to completion is called coulometric titration by Swift and associates (*187*). The end of the process may be noted by change in potential with a pair of additional electrodes, or by other methods—e.g., amperometric. Epstein and others (*61*) utilized the idea in the determination of acids by electrolytically generated hydroxyl. Oelsen and Göbbels (*144*) also used potentiometric indication in this type of process. Shaffer *et al.* (*188*) have developed an automatic instrument that electrolyzes mixtures of reagent and sample brought together in a constant ratio. The electrolysis current is increased or decreased in accord with signals fed back from a pair of indicator-reference electrodes. The electrolysis current may be indicated or recorded and calibrated in terms of concentrations of a particular substance in the sample. The principles of the coulometric method are admirably adapted to microdeterminations and to automatic indication or recording of content of a flowing solution or a gas stream.

Titrations in Partial Vacuum. Apparatus has been devised by Kunst and Sprengel (*109*) for carrying out potentiometric titrations under a pressure of about 16 mm. in a nitrogen atmosphere.

MICROTITRATIONS

Stock (*202*) has given an extensive review of the microchemical aspects of the dead-stop method (cf. Böttger, *18*). Frahm (*71*) discussed potentiometric methods in a general microchemical review. Ingold (*98*) attained accuracy, within ±5%, in the titration of 0.300 to 0.900 mg. of organic acids in a volume of 1 ml. The solution was placed in a re-entrant cup in a special glass electrode. Traces of acids or bases may be accurately titrated in unbuffered solutions, according to Kunst and Sprengel (*108*).

Syringe burets with revolution counters attached to the threaded drive shaft have been widely utilized in microtitrations.

CONCENTRATION CELL METHODS

Swain and Ross (*177, 205*) found potentiometric titration useful in kinetic studies. Two similar electrodes, connected through a galvanometer, were placed in separate beakers connected by a salt bridge, and a blank solution in one beaker was titrated rapidly with a reagent that simulated the conditions that developed in the other beaker. The amount of reagent necessary to balance the galvanometer was noted as a function of time. The hydrolysis of *tert*-butyl chloride and the oxidation of oxalate by ceric sulfate were studied in this manner.

DETAILED APPLICATIONS OF POTENTIOMETRIC TITRATIONS

Acid-Alkali Processes. IN AQUEOUS MEDIA. The variation of pH with dilution in equilibria involving hydrogen ions and systems such as chromate-bichromate, polyvanadates, etc., has been subjected to mathematical analysis by Carpeni and Souchay (*27*). Jordan and Taylor (*102*) have devised a correction for the alkali required by the solvent in the titration of weak acids and bases. Their considerations are useful in studies of polypeptides.

The galvanometric method with quinhydrone *vs.* reference electrode, a high resistance, and a galvanometer has been utilized by Eustigneev (*62*) and Gorbacheva (*81*) for the determination of acids in colored media.

Bereau and Tous (*10*) have made studies of the further applicability of the quinhydrone and antimony electrodes in the titration of acids. Weiner and Mahr (*223*) reported that copper and nickel interfere with the use of quinhydrone electrodes in the titration of acids. Kauko and Komulainen (*105*) estimated carbonate alkalinity in water by direct pH measurement. Anderson and Robinson (*2*) derived a table of activity coefficients, f_{H^+}, to be used in connection with the determination of the alkalinity of sea water by titration of acid, where pH $= -\log C_{H^+} f_{H^+}$. Carbon dioxide and a small amount of hydrochloric acid in distilled water may be estimated by titration with barium hydroxide, according to Cuta and Kohn (*45*), and the data indicate the existence of barium bicarbonate.

Giraut-Erler (*80*) found that weak acids at very low concentration cannot be titrated accurately using a glass electrode.

The titration of boric acid has been reinvestigated potentiometrically by Ruehle and Schock (*178*). The boric acid complexes with mannitol with one and two molecules of mannitol per molecule of borate have association constants $K_1 = 3 \times 10^2$ and $K_2 = 5.1 \times 10^4$, respectively, and according to Deutsch and Osoling (*51*) these anionic complexes persist in alkaline solution.

Eitel (*59*) has studied the titration of acetone, acetaldehyde, *o*-chloro- and *o*-nitrobenzaldehydes, furfural, and salicylaldehyde with hydroxylamine hydrochloride using the glass electrode-calomel cell.

The well known change in pH that occurs in the titration of sulfate by barium chloride solution may be followed with either an antimony or a quinhydrone electrode (Sierra and Carpena, *192*).

Hypochlorite may be titrated in the presence of sodium hydroxide with standard acid if contamination due to carbon dioxide is avoided, according to Rodriguez (*176*).

Molybdates. Bye (*25*) states that in the titration of sodium molybdate with hydrochloric acid the heteropoly anion may be $[H_7Mo_6O_{24}]^{----}$ or $[H_8Mo_7O_{28}]^{------}$. Carrière and Guiter (*28*) found that basic salts are formed at high pH and acid salts at low pH in the titration of molybdic acid or molybdates in the presence of alkaline earth or lead ions. Carpeni (*26*) found that the presence of neutral salts decreased the first break in the titration curve of molybdic acid. The breaks were at 0.5 and 2 equivalents of base per gram-atom of molybdenum.

Precipitation of Hydrous Oxides. Numerous new studies of the course of pH during the titrations of various salt solutions have been made. Yadava and Chatterji (*228*) followed the stability of colloidal solutions of aluminum and ferric hydroxide sols potentiometrically.

Moeller and Kremers (*134*) found the following basicities of rare earth hydroxides relative to that of yttrium taken as unity: lanthanum 1235, cerium 185, praseodymium 333, neodymium 23.5, samarium 8.4, europium 4.2, gadolinium 2.6, erbium 0.16, thulium 0.041, ytterbium 0.036, and lutecium 0.031.

Moeller and Rhymer (*135*) studied the precipitation of cadmium from the nitrate, chloride, bromide, iodide, and sulfate. Excess potassium iodide, bromide, or chloride may inhibit the precipitation completely. Carrière *et al.* (*29*) studied the precipitation of cadmium sulfate with sodium hydroxide. Quintin (*163*) measured the concentration of both hydrogen and cadmium ions during the precipitation of cadmium sulfate by potassium hydroxide. Precipitate and solution were separated after various times of treatment. Time of contact of solution and precipitate was found to have an important bearing on the pH of the solution at various stages in the treatment.

Copper. Brouty (*22*) found evidence of the formation of hydrated $CuSO_4.3Cu(OH)_2$ in the potentiometric titration of copper sulfate. Guiter and associates (*30*) made detailed studies of the precipitation of copper from solutions of the sulfate, nitrate, and chloride. Basic salts of the type $MX_2.3CuO$ and $CuSO_4.9CuO$ were reported.

Bordoni (*20*) studied precipitation methods for the determination of copper, iron, aluminum, zinc, nickel, chromium, and lead ions.

Geloso and Fauchere (*78*) reported evidence of the existence of $Pb(NO_3)_2.Pb(OH)_2$, $Pb(NO_3)_2.3Pb(OH)_2$, and a more basic salt, possibly $Pb(NO_3)_2.9Pb(OH)_2$ on the basis of titrations of lead nitrate with sodium hydroxide.

Various basic salts of uranium were reported by Guiter (*88*) in the titration of uranyl nitrate with sodium hydroxide. Uranyl acetate and sodium hydroxide gave a first inflection corresponding to uranyl hydroxide and a second corresponding to $Na_2O.8UO_3$ in Guiter's (*89*) experiments.

Guiter (*87*) studied the titration of vanadic acid solutions with alkali in the presence of alkaline earth and lead ions.

The reporting of basic salts on the evidence of titration data should be viewed with caution, for in many cases equilibrium conditions are not established.

Näsänen (*142*) found that the precipitation of zinc hydroxide proceeds stoichiometrically in the presence of sodium, barium, or calcium ions, but that magnesium and sulfate ions interfere. Carrière *et al.* (*31*) found that a correct determination of zinc could be made by titration of chloride or nitrate solutions of zinc with sodium hydroxide. They found a basic salt, $ZnSO_4.ZnO$, at pH 7.6 to 9.2 in sulfate solutions; it changed to zinc hydroxide at pH 9.4 to 11.5

IN NONAQUEOUS MEDIA. Wolff (*227*) has pointed out that relative strengths of acidic or basic functions depend on both the charge type and the acidic or basic properties of the solvent. Uncharged acids (hydrofluoric, nitric, RCOOH) are of relatively constant strengths in various media, whereas positively charged acids such as hydrated cations, NH_4^+, etc., increase in acid strength as the dielectric constant of the medium decreases.

Lykken and associates (*122, 124*) have made extensive studies of titrations with the glass electrode as indicator in nonaqueous media with special reference to highly colored mixtures that are of interest in petroleum researches. The general technique and details of procedure are of considerable interest in all work with nonaqueous media. A benzene–isopropyl alcohol mixture in 1 to 1 ratio with about 0.5% of water was found to be of general utility.

Anhydrous acetic acid as a medium continues to be useful. Kilpi and Puranen (*106*) found 2.8×10^{-13} for the self-ionization constant. Tomicek (*210*) made satisfactory titrations of sulfonamides in this medium. Wittman (*226*) titrated basic nitrogen in oils with perchloric acid in this medium.

Virasoro (*219*) found that fatty acids in butanol may be titrated satisfactorily using the glass electrode. Izmailov and Tartyllo (*101*) found that sodium or potassium acetates may be titrated much more satisfactorily in alcoholic media than in water.

Palit (*146*) utilized the solubilizing properties of glycol-hydrocarbon solvents for many interesting types of titrations. A glycol plus butanol, isopropyl alcohol, amyl alcohol, dioxane, or chloroform is effective for the titration of salts of fatty acids, boric acid or borax, ammonia, aniline, etc. A glass electrode is used as indicator.

Dioxane-water mixtures containing 50 to 65% of the former were used by Gale and Lynch (*76*) in making careful titrations of various dibasic acids in solutions of constant ionic strengths. The ionization constants of oxalic, malonic, succinic, and glutaric acids were derived from the data. The quinhydrone electrode was used.

A new direction has been given to research in the nonaqueous field by work of Moss *et al.* (*136*), who studied titrations in ethylenediamine. The sodium salt of ethanolamine was used as a base. The medium is suitable for differentiating between carboxylic and phenolic functions in resins. Amino acids give satisfactory titration curves. Either an antimony or a hydrogen

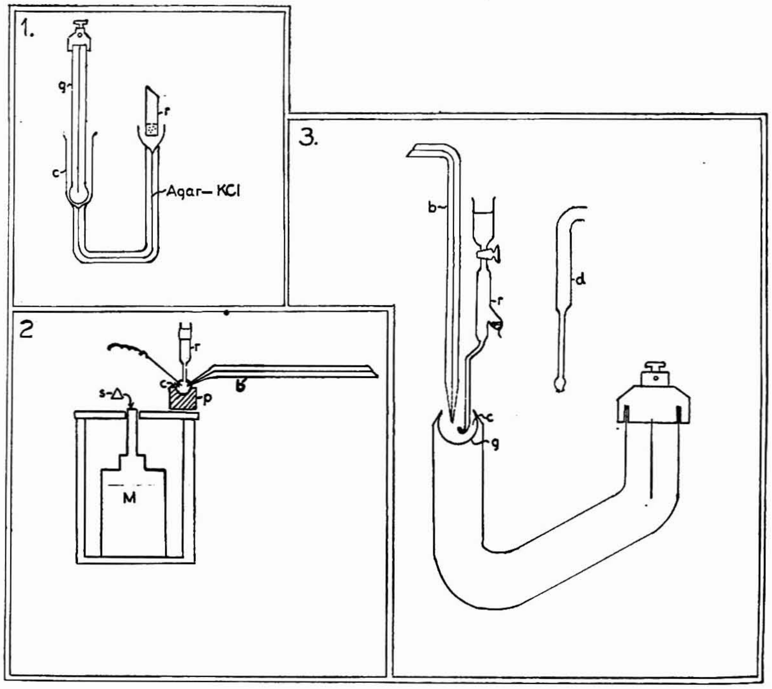

Figure 2. Apparatus for Potentiometric Microtitrations

1. Apparatus of Catch, Cook, and Kitchener (*34*). Glass microelectrode *g* makes contact with 0.1 to 0.2 ml. of solution containing 2 to 5 mg. of acid or base. *r* is tip of reference electrode.

2. Apparatus of Zürcher and Hoepe (*234*) for titration of halide in single drop of liquid in depression on paraffin block, *p*. *b* buret, *r* reference electrode, *M* motor, *s* triangular section on motor shaft to vibrate solution in *c*. Silver wire indicator electrode is used.

3. Apparatus of Ingold (*98*) for microtitration of acids. *g* is re-entrant glass membrane, *c* titration cell, *b* microburet, *r* reference electrode, *d* tube for withdrawing solution from fragile cell.

electrode may be used, but the glass electrode is unsatisfactory in this medium.

Schaal and Rumpf (*185*) have studied titrations in nitrobenzene.

Peterson *et al.* (*154*) proved that acids such as sulfur trioxide, ferric chloride, or stannic chloride may be titrated potentiometrically with bases such as quinoline, isoquinoline, or pyridine in an anhydrous selenium oxychloride medium.

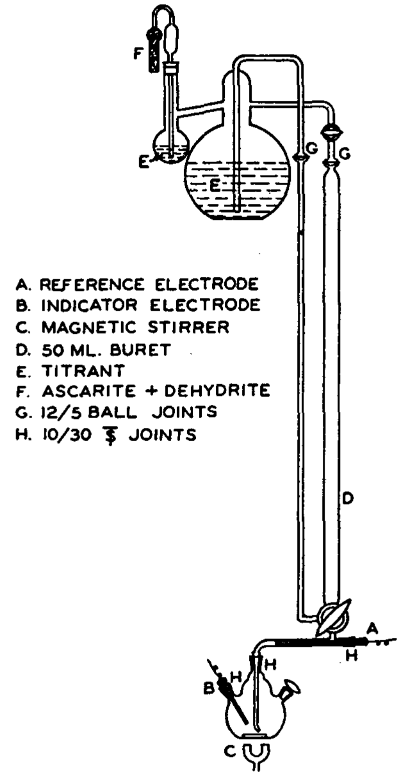

Figure 3. Titrations in Ethylenediamine as Solvent (*136*)

Precipitation and Complex Formation (see also hydrous oxide processes).

PROCESSES INVOLVING SILVER OR HALIDES. Scott (*186*) set up a calibration graph that was approximately linear for the estimation of chloride in water from 1 to 1000 p.p.m. The electrode system was silver–silver chloride solution to be tested and a 2 *N* magnesium sulfate–lead sulfate–lead amalgam. A similar idea was proposed by Mitoff and Schaaf (*133*) using the cell silver–silver chloride solution in a calomel half-cell. The calibration line shows appreciable curvature. Gilbert (*79*) and Riedel (*167*) have also used the silver–silver chloride electrode in measurements of small concentrations of chloride ion.

The well established idea of using a quinhydrone electrode at proper pH and a silver electrode to give zero e.m.f. at the end point of the titration of chloride with silver nitrate has been restudied by Rocha (*175*). Dean and Hawley have developed a portable apparatus for field determinations of chloride and dissolved oxygen in water (*47*).

The microtitration of chloride accurate to 0.02 ml. of 10^{-4} molar silver nitrate has been described by Northrop (*143*).

Simplified circuits have been applied to the determination of chloride (Zhidkikh, *232*) or in magnesium and its alloys (*217, 218*).

Yeck and Kissin (*230*) used the cell silver solution–potassium nitrate saturated with silver chloride, silver chloride–silver, and a vacuum tube voltmeter in the titration. Other modifications that have been recently utilized in the determination of chloride are: a silver amalgam indicator electrode (Chirkov, *41*); titration to a definite potential, in the estimation of chloride in copper solutions (Yao, *229*); successive titration of oxalate and chloride (Shchigol and Birnbaum, *190*); chloride in presence of sulfur dioxide (Berkovich and Luzina, *12*); and chloride in presence of bromide and iodide in ammoniacal medium (Shchigol, *189*). Three breaks occur, one at the end of the precipitation of iodide, a second at the end of the bromide precipitation, and a third at the end of the formation of $AgCl(NH_3)_2$.

Calcium may be estimated indirectly in the presence of magnesium by precipitation with standard oxalate solution, filtration, and titration of the excess oxalate with silver nitrate, according to Birnbaum and Shchigol (*17*).

Sodium salts of lauric, myristic, palmitic, or stearic acid may be titrated potentiometrically with 0.02 to 0.1 *N* silver nitrate (Ekwall and Juup, *60*). Davies and Armstrong (*46*) have confirmed the fact that mercaptan (thiol) in the presence of sulfides may be titrated with silver nitrate. Phosphate may be determined indirectly by precipitation with excess silver nitrate in a borax buffer of pH 9, adjustment to pH 7 to 8, filtration, and titration of excess silver ion with standard potassium bromide solution (Flatt and Brunisholz, *67*).

Photographic developer solutions may be analyzed potentiometrically for bromide [Crowell *et al.* (*44*) and Stott (*203*)] as well as for sulfate (*203*). Plasencia (*155*) separates silver and halides in emulsions by alkali, and titrates the silver and the halides separately.

Bellamy (*8*) has reinvestigated the titration of silver with thiocyanate solution.

Cyanide Processes. Gregory and Hughan (*83*) estimate the silver in plating solutions by titration with cyanide. Chirkov (*40*) used the platinum-silver electrode pair in titrating copper or nickel in solutions of their ores with cyanide solution. The total cyanide in potassium cuprocyanide solutions may be titrated with mercuric nitrate, using an amalgamated platinum electrode as indicator. Free cyanide is estimated by titration with zinc chloride to a turbidity end point, according to Weiner (*222*). Gaguin (*74*) found $10^{-11.4}$ for the solubility product of silver cyanide and 0.395 volt for the E_0 value of the process: $Ag(CN)_2^- + e = Ag + 2CN^-$, and 0.152 volt for that of $Ag[Ag(CN)_2] + e = Ag\ (CN)_2^- + Ag$. The titration of nickel with cyanide has been reinvestigated by Chirkov and Noranovich (*42*).

Fluoride Processes. The titration of aluminum with fluoride solution, using the ferri-ferrous indicator electrode, has been applied to ores and silicates by Stefanovskiĭ and Svirenko (*200*), to magnesium alloys by Mannchen (*131*), to electron-type alloys by Pollak (*157*), and to bronzes and steels after prior removal of interfering elements by Ivanov and Bezyaiko (*99*).

Similar methods apply to the determination of beryllium (Tarayan, *208*) and calcium (Tarayan, *209*), and to the precipitation of lead as the chlorofluoride (Farkas and Uri, *64*). The determination of fluoride by precipitation with ferric chloride according to Treadwell's procedure has been utilized by Talipov and Teodorovich (*206*).

Ferrocyanide Precipitations. New studies have appeared on the titration of various metallic ions with standard potassium ferrocyanide solutions:

Cadmium. Bhattachary and Gaur (*13*), Berkovich (*11*), Kale (*104*)
Copper and Lead. Kale (*103*)
Nickel. Tannaev and Levina (*207*)
Thallium as $CaTl_2\ Fe(CN)_{6\ 2}$. Ripan and Poppev (*170*)
Zinc. Shistermann and Kolesnikova (*191*); in aluminum alloys, Eisen (*58*); in bronze after removal of copper, tin, lead, and iron Pchelintsen (*148*)

Miscellaneous. Lead may be determined indirectly by adding an excess of a standard solution of potassium iodate; the excess of the latter is determined with silver nitrate solution, according to Dragulescu and Latiu (*52*). Näsänen has studied the solubility of lead iodide in solutions of various perchlorates (lithium, sodium, barium, cadmium), and nitrates (lithium, sodium, barium), and in sodium chloride by potentiometric technique (*141*). The methods of preparation of electrodes of silver–silver chloride and silver–silver sulfide have been studied by Martinez (*132*). According to Druet (*53*) the variation in the end point in the determination of silver depends upon the age and nature of the electrode.

When ferric chloride is added to sodium monohydrogen phosphate solution, ferric phosphate forms directly, according to Hubicki and Sykut (*95*), whereas in the reverse process $2FePO_4 \cdot Fe(OH)_3$ is formed.

Oxidation-Reduction Processes. The work in this field is summarized according to individual reagents arranged roughly in order of descending oxidizing power. Applications to the analysis of precious metals and to the steel and ferroalloy fields are collected at the end of this section.

Ceric Sulfate. Copper(I) and antimony(III) may be titrated successively after prior reduction with chromous chloride (Pribil and Chebovsky, *162*).

The arsenic(III)-cerium(IV) reaction may be used for indirect catalytic estimation of iodine, according to Hahn (*93*).

Cerium(IV) may be titrated with ferrous ion for the estimation of cerium in its alloys with iron (Raisin-Streden and Müller-Gamillschweg, *164*) and in steels (Malov *et al.*, *130*).

Potassium Permanganate. Pappas (*147*) found that acetate improves titration of uranium(IV) in the presence of iron(II).

Manganese (II) is oxidized to tripyrophosphatomanganic(III) ion, $Mn(H_2P_2O_7)_2^{---}$, by potassium permanganate at pH 6 to 7. Chloride and ions of cobalt, iron, chromium, copper, nickel, wolfram(VI), molybdenum(VI), uranium(VI), zinc, aluminum, magnesium, and cadmium do not interfere according to Lingane and Karplus (*118*). The reverse titration of manganese(III) pyrophosphate ion with iron(II) may be used for the estimation of manganese (Watters and Kolthoff, *221*).

Manganese(II) may be oxidized to manganese(III) in the presence of fluoride. Zvenigorodskaya and Gotsdiner (*236*) applied this process to ores and slags.

Treadwell and Nieriker (*215, 216*) have made a general investigation of the production of the ions of chromium of (II), uranium(III), uranium(IV), wolfram(IV), wolfram(V), and molybdenum(III) by reduction with cadmium, followed by oxidimetric titration.

Indicators. The formal potentials of *o*-phenanthroline and bipyridine-ferrous complexes have been redetermined (*97*).

Potassium Bichromate (see also analysis of steels). Oxford (*145*) has restudied the titration of iron(II). Zhivanovich (*233*) uses a platinum–ferric chloride reference electrode in bichromate titrations.

Hydrogen Peroxide was used by Gazulla (*77*) in the estimation of nitrites.

Lead Tetraacetate was used by Reeves (*165*) to estimate *cis*-glycols, the electrodes being lead *vs.* platinum.

Potassium Bromate. Arsenic(III) and antimony(III) were determined with bromate by Smith (*195*) using the polarized platinum-platinum electrode system.

Thallium(I) may be titrated in presence of iron(III) complexed by phosphate on a micro scale according to Rienäcker and Knavel (*168*).

DuBois and Skoog (*54*) determined bromine numbers with standard potassium bromite–bromate solution and mercuric chloride catalyst.

8-Hydroxyquinoline may be determined by the addition of excess standard bromate followed by back-titration with arsenite (Savioli, *184*).

The platinum–platinum black electrode combination was used by Bielenberg and Kühn (*14, 15*) in bromination of phenols.

Hypobromite was used by Chirkov (*37*) to titrate phenol.

Bromate, Bromine, and Bromide mixtures may be examined by titrating free bromine with aniline sulfate, then adding excess of antimony(III) standardized solution, and titrating the excess with standard potassium bromate solution (Portnov and Elkina, *161*).

Hypochlorite. Maksimyuk and Ptitsyn (*129*) have investigated the reaction between thiosulfate and hypochlorite.

Potassium Iodate has been used by Spacu and Spacu (*196, 197*) for the indirect determination of thorium or of lanthanum (*198*). Singh and Rehmann (*194*) used potassium iodate in the direct estimation of aromatic amines. Spacu and Spacu (*199*) used the reagent for the estimation of *l*-ascorbic acid.

Ferric-Ferrous Reactions. Ferric chloride or sulfate solutions have been found useful by Rius and Coronas (*172*) in the estimation of molybdenum(III), or of molybdenum and vanadium (Rius and Martin, *173*). Lannet (*111*) applied the method to titanium, and Weiss and Blum (*224*) to uranium(IV).

Ferrous sulfate as reagent is used most frequently in steel analysis. Strouts and MacInnes (*204*) determined nitric acid in mixed acids with ferrous sulfate using a platinum–potassium iodate reference electrode. McKinney *et al.* (*128*) developed a similar method for estimating nitric acid in oleum.

Iodine-Iodide Reactions. Reagents containing iodine have been used in the indirect determination of silver in micro or submicro amounts, based on the reaction $2Ag + I_2 = 2AgI$, followed by titration of excess iodine with arsenite, using the dead-stop method (Lambert and Walker, *110*).

Sillars and Silver (*193*) determined oxygen in water by the indirect iodine-thiosulfate procedure.

Bates *et al.* (*7*) determined the amylose and amylopectin content of starch by standard iodine solution.

Water by the Karl Fischer technique is best estimated with the aid of the dead-stop end point as suggested by Wernimont and Hopkinson (*225*) and by Carter and Williamson (*33*). Rennie and Markham (*166*) applied the method to gunpowder, glacial acetic acid, sawdust, and shellac. McComb (*127*) applied it to protein material, and Gukhman *et al.* (*90*) studied various applications.

The following titrations with standard potassium iodide have been investigated: chloramine, by Afanasev (*1*); copper, applied to indirect estimation of iodine, by Hahn and Adler (*94*); osmium in osmic acid, by Ryabchikov (*180*); and selenates, by Ripan and Dragulescu (*169*) and de Salas (*183*).

Ferri- and Ferrocyanides as Reagents. Major attention has been given to the estimation of cobalt(II) by titration with ferricyanide. Chirkov (*39*) studied this method and the cyanide method for nickel and cobalt. Ivanova and Malov (*100*) and Bagshawe and Hobson (*3*) studied the application to steels. The latter (*3*) consider the formal potentials and the interferences of manganese chromium(III), and vanadium(IV). Zvenigorodskaya (*235*) avoided the interference of manganese by oxidizing it to manganese(III) in presence of fluoride with potassium permanganate. The standard ferricyanide solution was then titrated with the solution of the alloy.

Podlubnaya and Bukharov (*156*) estimate sugars by using their solutions to titrate alkaline ferricyanide solution.

Murgulescu and Dragulescu (*138*) found that hexammine cobaltic salts may be used to titrate ferrocyanide.

Cupric Ion. Britton and Clissold (*21*) used cupric reagent for the titration of phenyl hydrazine.

Potassium Mercuric Iodide, K_2HgI_4, may be titrated with arsenite or antimonite in alkaline medium (*72*).

Molybdenum(V) as Reductant. Tourky *et al.* (*212*) studied the stability of the solution and the titration of iron, and also (*213*) titration of iodate, bromate, bichromate, and vanadate. They later (*214*) investigated the titration of various mixtures of oxidants. Rius and Coronas (*171*) followed potentiometrically the interaction of molybdenum(VI) and molybdenum(III) to yield molybdenum (V).

Thiocyanate. Gaguin (*75*) studied the reducing properties of thiocyanate ion at various pH ranges.

Stannous Chloride was used by Vasilev (*217*) for the indirect estimation of phosphorus by titration of molybdiphosphoric acid.

Vanadous Sulfate has been applied by Banerjee (*4, 5*) to the titration of titanium and to the estimation of organic substances.

Chromous Solutions have been the subject of investigation.

Apparatus and application of chromium(II) to the estimation of molybdenum, wolfram, uranium, vanadium, titanium, antimony, tin, and bismuth. Flatt and Sommer (*68*).

Estimation of wolfram and vanadium. Flatt and Sommer (*69*).

Estimation of iron, copper, titanium, vanadium, chromium, molybdenum, and wolfram. Flatt and Sommer (*70*).

Titration of heteropoly acids of silicon and phosphorus Tourky and El Shamy (*211*).

Estimation of wolfram. Chernikhov and Goryushina (*36*); (*68–70*).
Iron and copper in alloys. Pollak (*158*); (*70*).
Selenium and tellurium. Lingane and Niedrach (*119*).

2,6-Dichlorophenolindophenol. Bogdanuva (*19*) and Liebman and Ayres (*113*) used this reagent for the titration of vitamin C.

Applications in Steels and Ferroalloys (see also ferricyanide process for cobalt, reductions with chromous solutions, oxidations with ferric solutions).

Zanko (*231*) has given a general review on chromium, manganese, vanadium, molybdenum, titanium, and iron.

Chromium and Vanadium. Numerous adaptations of the well-known process for the estimation of chromium plus vanadium after prior oxidation to chromium(VI) and vanadium(V) by titration with ferrous sulfate have been made. A list of the investigations and some idea of the similarities and differences are given in the following summary:

Method of Oxidation Prior to Cr plus V Titration	Method of Destroying Excess Oxidant Prior to Titration with Fe(II)	Investigators
Persulfate—Ag	NaCl (after $KMnO_4$ oxidation) in reoxidation of V(IV)	Grenberg and Genis (*84*)
	$C_2O_4^{--}$	Sabinina and Liventseva (*182*) L'vova (*121*) Claassen and Corbey (*43*)
	MnO_4^- titrated with $C_2O_4^{--}$. Titrate V(V) and Cr(VI) Reoxidize V(IV) with MnO_4^- destroy excess with $NaNO_2$	Bengolea and Costa (*9*)
	MnO_4^- destroyed with NaN_3	Birckel (*16*)
$KMnO_4$	Excess destroyed by oxalate	Gottardi (*82*)
$KMnO_4$	Excess destroyed by oxalate	Chateau (*35*)
Nitric acid	....	Volkov (*220*)

Estimation of Platinum Metals. The rather numerous investigations in this field are summarized briefly:

Substances Titrated	Reagents and Other Data	Reference
$(NH_4)_2PtCl$ or $(NH_4)_2IrCl_6$	Cuprous chloride	Ryabchikov (*179*)
$(NH_4)_2IrCl_6$	Ferrous sulfate	Kozyar (*107*)
$(NH_4)_3IrCl_6$	Permanganate	Kozyar (*107*)
$PtCl_2$ or $IrCl_2$	Permanganate	Grindberg *et al.* (*86*)
Tris 1,10-phenanthroline ruthenates	Formal potential 1.29 volt (*N* HNO_3)	Dwyer *et al.* (*55*)
Os(IV)-Os(III) in HBr	0.452 volt at zero acid	Dwyer *et al.* (*56*)
Os(IV) - Os(III) in HCl	0.452 volt at zero acid (extrapolated)	Dwyer *et al.* (*57*)
Pt(II)	Ceric sulfate	Ryabchikov (*181*)
Pt(IV)	Cuprous chloride	Ryabchikov (*181*)
Au(III)	Hydroquinone; $FeSO_4$	Ryabchikov (*181*)
Ir(IV)	Hydroquinone	Ryabchikov (*181*)
Os(VIII)	KI, or $Na_2S_2O_3$	Ryabchikov (*181*)
Pd(II) to PdS	$Na_2S_2O_3$	Ryabchikov (*181*)
Platinum complexes with amines or glycine	Formal potential 0.630–0.691. $PtCl_6^{--} \rightleftharpoons PtCl_4^{--}$ 0.785 volt in NaCl	Grindberg and Lavrent'ev (*85*)

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CHROMATOGRAPHY

HAROLD H. STRAIN, *Argonne National Laboratory, Chicago, Ill.*

WITH the development of the natural sciences, man has based his understanding of the universe upon the properties of inorganic and organic matter. Among all material substance ranging from the several forms of energy to the intricate associations of living organisms, there are various degrees or levels of complexity. These organizational levels may now serve for the comparison and classification of matter and for evaluation of the advancement of knowledge. With some oversimplification and with certain arbitrary assumptions, the relative complexity of most substance may be summarized in tabular form as illustrated by Table I.

The relationships among matter at the different organizational levels are more complex than the relationships among matter at each level. Although there are many opportunities for progress in the understanding of highly organized material substance in terms of less complex matter, most investigations and interpretations have been confined to substance at a single organizational level. This limitation to the progress of knowledge can be attributed to various conditions such as the extreme specialization of scientific investigators (*10*), the limitations of man's sensitivity and perception, and the limitations of scientific methods.

Table I. Relative Complexity of Inorganic and Organic Substance

Increasing Complexity	Substance
↓	Various forms of energy: the basic unit of radiant energy, the photon; the carrier of kinetic energy, the neutrino (massless particles)
	Basic units of electricity and of matter: the electron and the positron (lightest particles)
	Intermediate particles: the mesons
	Basic components of atomic nuclei: the proton and the neutron
	Chemical elements: hydrogen to curium
	Chemical compounds: inorganic to organic; minerals including coal and petroleum
	Rather constant associations of chemical compounds: soil, air, sea water
	Particular, improbable associations of labile compounds: living cells
	Associations of living cells: multicellular organisms, reproductive groups
	Complex associations of multicellular organisms: colonies, hives, flocks, herds, tribes, cities, and nations

Recognition of most of the degrees of complexity in nature has resulted from the use of sensitive instruments and of specialized analytical techniques (*27, 38*). With a few notable exceptions, these methods of investigation have proved most effective with matter at but one level of complexity.

APPLICABILITY OF CHROMATOGRAPHIC ANALYSIS

Of the methods devised for the examination of matter, few have found such wide application under such a variety of conditions as chromatographic analysis. Invented in different modifications by Schönbein, by Goppelsroeder, and by Tswett and improved and applied by many other workers, this method has continued to yield significant new results in many diverse fields (*14, 28, 30, 69, 118, 119*). It has proved useful with substance varying enormously in degree of complexity—as for example, the chemical elements, various chemical compounds, microscopic parts of cells, and even the cells themselves (*105, 118*).

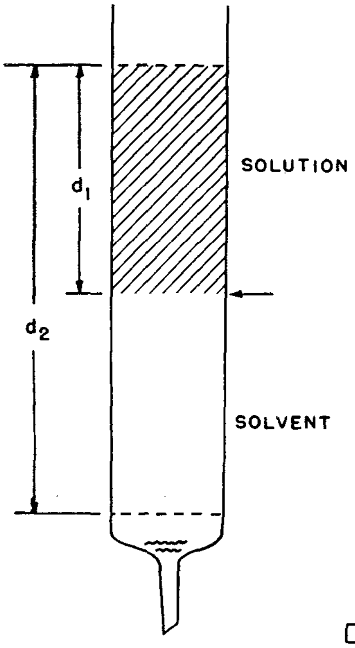
Chromatographic analysis is especially effective for the resolution of mixtures and for the isolation and identification of the components. As the resolved materials may be isolated without chemical change, chromatography has become a basic tool for all scientists whose advance into unexplored regions depends upon knowledge of the properties of particular substances (*119*).

Because of the discovery and improvement of chromatographic methods in remotely related fields, evaluation of the technique with respect to applicability to new materials has been difficult and time-consuming. Progress in this direction has been facilitated by reviews that have appeared from time to time (*28, 30, 118*). An extensive summary with 538 literature citations covering applications of chromatographic analysis in organic chemistry for the years 1939–1949 has been prepared by Lederer (*69*).

Another review devoted to the properties of ion exchangers, which have been utilized for many chromatographic separations of inorganic ions, has been prepared by Nachod and fifteen collaborators (*87*). A general summary of chromatographic techniques, first presented several years ago as part of the lecture series, "Frontiers in Chemistry," has also been brought to publication (*119*). Advances in the use of chromatographic methods for following the chemical and metabolic transformations of carbon compounds labeled with isotopic carbon have been reviewed by Calvin and co-workers (*5, 18, 19*). Numerous symposia in Europe and in the United States have also helped to disseminate knowledge about this useful technique (*3, 126*). The indexes of *Chemical Abstracts*, of *Annual Reviews of Biochemistry*, and of many scientific journals have been expanded to include various applications of the chromatographic technique. In view of the availability of most of this material, no attempt has been made to report here all the recent research or industrial applications (*8, 113*). Instead, emphasis has been placed upon the nature, the mechanism, and the modifications of chromatographic analysis.

NATURE OF CHROMATOGRAPHY

There has been a gradual clarification of the methods and procedures which are now recognized as chromatographic analysis. This technique depends upon a dynamic partition or distribution of dissolved or dispersed substances between two immiscible phases one of which is moving past the other (*87*, *119*).



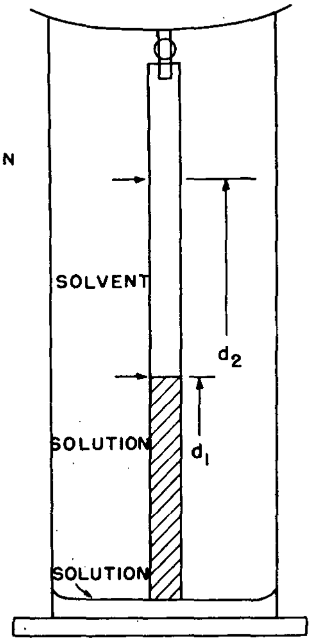


Figure 1 (*Left*). Filtration of Solution through an Adsorption Column
Figure 2 (*Right*). Ascent of Solution in Strip of Filter Paper

Distance moved by solution, d, and by solvent, d_2

In practice, chromatographic analysis involves a particular, discontinuous procedure. The mixture to be resolved is absorbed in an absorptive column (Figure 1), in one end of a strip of paper (Figure 2), at the side of a sheet of paper (Figure 3), or in a stack of filter paper (*85*), wherein it is washed with fresh solvent or with mixtures of solvents so that the solutes migrate through the porous, nonmobile phase at different rates and separate from one another. Although these physical arrangements may be varied in many ways, as by use of various absorptive phases and by use of various solvents, the principles involved in the chromatographic resolution of mixtures are few indeed (*69, 87, 119*).

PARTITION MECHANISM IN CHROMATOGRAPHIC SEPARATIONS

The dynamic partition of solutes between the two phases in chromatographic separations may be represented by equilibrium equations.

$$\text{Stationary phase} \leftrightarrows \text{Mobile phase}$$

$$\begin{tikzcd} & \text{Solutes} \arrow[dl, shift left] \arrow[dr, shift left] & & & \text{Solutes} \arrow[d, shift left] \\ \text{Solvent} \arrow[ur, shift left] \arrow[rr, shift left] & & \text{Adsorbent} \arrow[ul, shift left] \arrow[ll, shift left] & \leftrightarrows & \text{Solvent} \arrow[u, shift left] \end{tikzcd}$$

These equilibria may involve solution or dispersion of the solute. They may involve ionization, solvation, or association of the solute. There may be complex formation with the phase itself or with added substances. There may be a variety of reversible chemical reactions, and in the stationary phase, there may be various surface-adsorption phenomena. Meinhard (*82*) has shown that several combinations of reversible systems remain to be examined as a basis for chromatographic separations. In his sixteen arbitrary combinations, adsorption of the solute and solution of the solute in the stationary phase are placed in the same class, although for practical reasons they are usually considered separately (*119*).

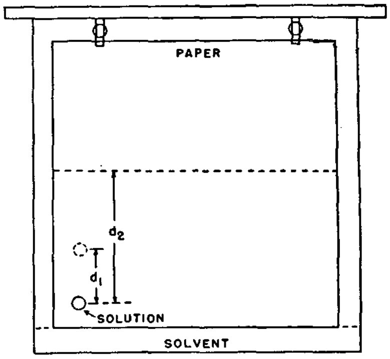


Figure 3. Solution in Paper Washed with Fresh Solvent as in Formation of Chromatogram

Even though certain of these reversible phenomena may predominate in a single absorption system (*119*), most of the attractive forces are usually present in varying degree. Recent data on the absorption of organic acids by ion exchangers suggests that the principal reaction between the acid and the insoluble amine resin may vary with the solvent, ion exchange predominating in the presence of water, addition reactions predominating in the presence of nonaqueous solvents (*107*).

In absorptive filter paper, the resolution of mixtures of solutes may depend upon surface adsorption, upon ion exchange, or upon partition between solvents (*69, 118, 119*). The predominant force varies with the paper, with the solvents, and with the solutes. Both paper and siliceous filter aids may be impregnated with various adsorbents, ion exchangers, and chemical reagents (*57, 133*) so that the attractive forces are varied enormously, whereas the desirable physical properties of the support are retained.

With absorptive solids, the predominant attractive forces may be chemical reactions, such as ion exchange and complex formation; surface attractions, such as the adsorptive forces of surface-active solids; and solution, such as the dissolution of solutes in the liquid retained by a gel or moist solid (*107, 119*). The amount of solute retained by the solid phase in relation to the

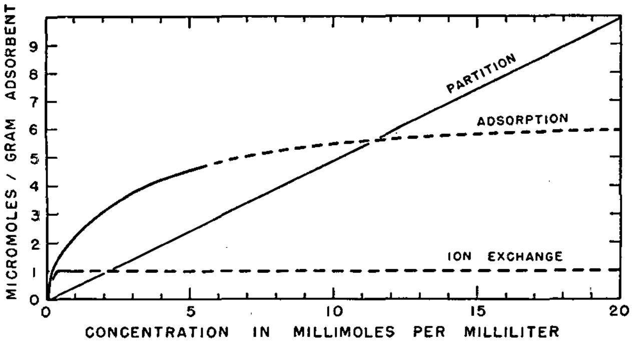


Figure 4. Isotherms Representing Absorption of Silver Ion by Ion Exchanger (*11*), Adsorption of Acetic Acid by Bone Charcoal, and Partition of Solute between Two Immiscible Liquids

Dashed portions of curves represent extrapolations

concentration of the solute when these different forces predominate is illustrated by the curves in Figure 4.

VARIABLE CONDITIONS IN CHROMATOGRAPHIC SEPARATIONS

Numerous modifications of the chromatographic technique which have been reported in the past year pertain to the use of absorptive paper; hence they are considered in a subsequent section devoted to paper chromatography. Similarly, variations of the absorbents are treated under sections devoted to chromatography by adsorption, by ion exchange, and by partition.

A variety of solvents and solvent mixtures has been employed for washing the absorbed solutes. These solvents range from the nonpolar organic hydrocarbons to very polar aqueous solutions. As indicated by the equilibrium equations and as illustrated by many new examples, the solvent must be selected in relation to the properties of the absorbent and the solute mixture (*69, 118*). For uniform, reproducible separations, even filtration must be obtained (*35*).

An increasing number of examples shows that many combinations of solvents and absorbents may be employed for resolution of one kind of solute—for example, mixtures of organic acids may be resolved by absorption in columns of ion exchangers with water or with organic liquids as solvents, a variation that may lead to inversion of the adsorption sequence (*107*). They may be separated in columns of silica gel with chloroform or iso-octane as solvent (*50, 103*), in columns of Celite plus strong sulfuric acid and with benzene or butanol as solvent (*55, 94*), in columns of charcoal with various solvents (*56*), in columns of alumina with various solvents (*37, 69, 136*), and in paper with water, aqueous phenol, or butanol as solvent (*21, 79*).

Many analogous examples of separations of amino acids performed with various combinations of absorbents and solvents have been reported in the current literature and summarized in *Annual Reviews of Biochemistry*. Even the solutes themselves may be altered as by conversion into various derivatives and complexes (*9, 21, 32*).

TERMINOLOGY

The dependence of most chromatographic separations upon a few distribution phenomena has led to widespread acceptance of such terms as chromatography by ion exchange, by partition, and by adsorption (*69, 118*). Newer terms are electrochromatography, indicating the use of electromotive potential in columns or in paper (*69*), and salting-out chromatography (*125*), indicating the increased adsorbability of a proteinaceous solute due to the addition of salt to the solution. Surface chromatography, recently suggested as a name for separations of solutes in thin layers of absorbents (*83*), is likely to be confused with the common chromatography based on the use of surface-active adsorbents.

For the initial passage of solution into a column or strip of paper, such terms as adsorption or absorption of the mixture, filtration of the solution, the initial adsorption, and zone formation have been widely employed. Passage of fresh solvent through the absorptive layer to separate the absorption zones, though commonly called development of the chromatogram, is now widely called washing the absorbed substances, irrigation with fresh solvent (*10, 79*), treatment with complexing agents (*59*), and formation of the chromatogram (*118*).

The separate regions in the absorptive material that contain the individual, resolved solutes have been called absorption or adsorption bands or zones. Because of the possible confusion with spectral absorption bands, these regions in the columns might preferentially be called zones rather than bands.

Irrespective of the partition mechanism, the resolution of mixtures in paper is commonly called paper chromatography, analogous to columnar chromatography. Resolution of mixtures in a sheet of paper by irrigation in one direction with one solvent (Figure 3) followed by transverse irrigation with another solvent is now called two-way chromatography (*11*), a contraction of the two-dimensional chromatography suggested by Consden, Gordon, and Martin, and an improvement on the cross capillary analysis, first proposed by Liesegang (*118, 119*).

Colorless substances separated by chromatographic adsorption are often rendered visible by addition of reagents to the chromatogram (*69, 119*), a procedure widely utilized in paper chromatography (*69*). Colorless substances may also be made visible by the addition of reagents to the absorptive phase (*16*) and to the wash liquid (*89*). Because of the parallelism between this production of color and the development of a photograph, formation of colored derivates of solutes separated by adsorption is widely called the development of the chromatogram, contrary to the older meaning of this term. By the sheer weight of usage, especially in the field of paper chromatography, this new meaning promises to displace the older one. It may be better, therefore, to call the washing of the absorbed solutes the formation of the chromatogram, and the production of color with reagents the development or revelation or ripening (*83*) of the chromatogram.

The term chromatography, which evolved from Tswett's *chromatographische Adsorptionsanalyse*, no longer applies solely to the separation of colored substances, nor does the method depend entirely upon adsorption phenomena. Moreover, this terminology leads to the anomalous colorless chromatogram (*69, 83*). In view of the widespread use of all chromatographic methods for the resolution of mixtures, a name indicative of resolution into the smallest components would be less ambiguous than a name indicative of color. From this point of view, eography might substitute for chromatography.

RATE OF MIGRATION

One of the most useful constants in chromatographic investigations is the R value, the rate of migration of a solute through the absorptive phase relative to the flow of solvent. Ever since Goppelsroeder's extensive investigations of the capillary ascent of organic and inorganic solutes into strips of filter paper (*119*), this migration constant has proved useful for the comparison and description of the adsorbability of various solutes. Eventually, the quantitative considerations of the mechanism of chromatography led to the deduction that the R value was the ratio of unabsorbed solute in the interstitial spaces to the total solute in the adsorption zone (*118, 119*).

In practice, migration rates are determined by filtration of a solution through the absorptive medium as indicated by Figures 1 and 2, or they are estimated from the movement of an adsorption zone in a column or in paper, as indicated by Figure 3. In these three figures $R = d_1/d_2$, or $R =$ (amount of solute in solution in the adsorption zone) $\div$ (the amount of solute in solution plus the amount adsorbed). With an elastic scale, R can be read directly from the chromatograms (*95*).

As R is determined for a given adsorbent and solvent, the principal variable in the measurements is the concentration of the solute. In order to compare R with concentration, R values were calculated from the sorption isotherms illustrated in Figure 4. In this calculation, the interstitial volume was assumed to be 0.1 ml. per gram of adsorbent. The results, summarized in Figure 5, show that R values depend upon the concentration of the solute and upon the shape of the sorption isotherm. With surface active adsorbents and with ion exchangers, the R value increases gradually with concentration of solute, but with partition absorbents the R value remains constant over a great range of concentration as has so often been pointed out (*119*). Owing to the small rate of change of R with concentration, the R value, determined at a few concentrations of solute, may not be a critical measure of the kinds of forces that bind a solute to an absorptive phase. Indeed, the R value must be regarded as the summation of all the absorptive or distribution phenomena that occur during the migration of the solute. At the trailing boundary of an adsorption zone, the variation of R with the decreasing concentration of solute may be correlated with a rapid and troublesome widening of the zones (*59, 119*).

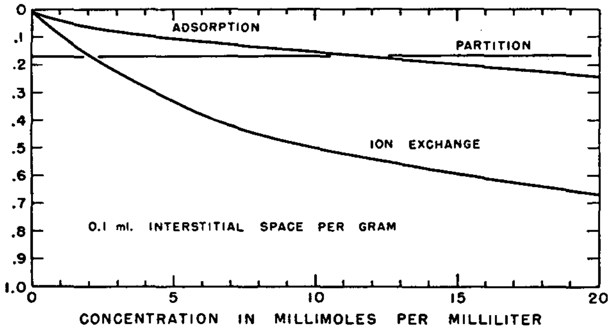


Figure 5. R Values, $R = d_1/d_2$, Calculated from Isotherms of Figure 4

Assuming 0.1 ml. interstitial space per gram of absorbent

If an absorptive phase is diluted with a nonabsorptive porous filter aid, the proportion of the solute retained in solution in the interstices will be increased relative to the total amount of solute present in the adsorption zone. With the adsorbent diluted until the interstitial volume is 1 ml. per gram of active solid, the R values calculated from Figure 4 are increased as illustrated by Figure 6. In comparison with Figure 5, these R values of the diluted surface-active and ion exchange absorbents increase more rapidly with concentration than the R values of the undiluted absorbent. Moreover, the R value of the diluted partition absorbent is increased by a constant amount.

From these examples, the variation of the R value with the concentration of solute provides a measure of the sorption capacity of the stationary phase. This variation also provides clues to the principal kinds of attractive forces in the nonmobile phase, it illustrates a convenient method for relating adsorption isotherms to chromatographic separations, and it shows the importance of the porosity of the absorptive phase in chromatographic separations.

ADSORPTION SEQUENCE

Long before the recent quantitative considerations of chromatographic methods (*69*), the sequence of substances adsorbed in columns proved useful for the description and identification of many labile compounds obtained from biological materials. Likewise, the mixed chromatogram, the chromatographic comparison of substances suspected of being identical, has been widely used as a sensitive descriptive and analytical test. In principle, these sequences represent qualitative comparisons of the R values. In practice they are extremely useful, because they are usually altered less than R values by the presence of impurities. Owing to their extreme sensitivity and because they can be seen during the preparation of many substances by chromatographic methods, adsorption sequences are certain to find further application in many fields. As the adsorption sequence may vary with the solvent and the adsorbent (*107, 118*), specification of the adsorption conditions is always essential (*121*).

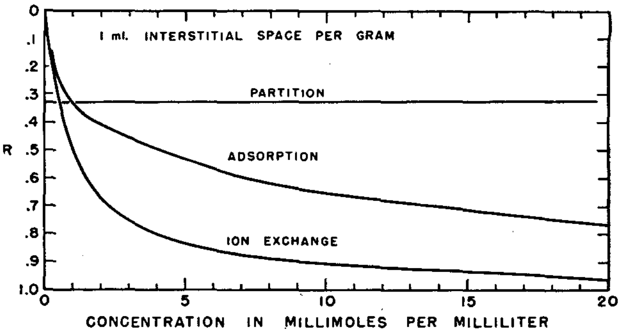


Figure 6. R Values Calculated from Isotherms of Figure 4

Assuming 1 ml. interstitial space per gram of absorbent

Adsorption sequences have been reported in several ways which reflect the analytical procedure but which are exceedingly confusing to the reader. When the flow of solvent is downward, as in columns (Figure 1) and in some uses of paper, the solutes are usually tabulated in the direction of decreasing adsorbability, that is, from the top to the bottom of the page (*62, 119*). But when the flow of liquid is upward, as in vertical sheets or strips of paper (Figures 2 and 3), the direction but not the order of the decreasing adsorbability is reversed. Tabulation of the results in this upward direction is convenient for the analyst, but the order of the adsorbability may not be clear (*48*). In such cases, ambiguity can be avoided only by indication of the direction of increasing or decreasing adsorbability.

LIMITATIONS OF CHROMATOGRAPHIC SEPARATIONS

Although much has been written about the great sensitivity and wide applicability of chromatographic methods, little consideration has been given to possible limitations of the technique. As information has accumulated, however, mixtures of certain kinds of substances have proved difficultly separable or inseparable in adsorption columns. An examination of the properties of these substances in relation to the mechanism of chromatographic analysis points to possible improvements of the separations.

In all chromatographic separations, there are two requisites: a dynamic partition or equilibrium of the solutes between the two phases, and a small difference among the relative amounts of each solute in the two phases. The dynamic equilibrium is easily achieved for nearly all kinds of solutes, but the difference in the partition coefficients of certain solutes may be too small for effective chromatographic separations.

Most information about the separability of various kinds of organic (*62, 69, 118, 119*) and inorganic (*59, 66, 87, 110, 111*) substances has been summarized in qualitative fashion in Tables II and III. From these tables, chromatographic separability is obviously related to differences among the polar or reactive groups of the solute molecules. These reactive groups are usually the most absorbed parts of the molecules; consequently, the separability of similar molecules is most effective when the immiscible phases attract those parts of the molecules in which the greatest structural differences reside (*118*).

Table II. Chromatographic Separability of Organic Compounds in Relation to Variations in Structure

Structural Variation	Separability
Molecular size	Fair to poor
Structural isomers	
Chains *vs.* rings	Fair to poor
Branched chains	Fair to poor
Position of double bonds	Fair to poor
Spatial isomers	
Cis-trans at double bond	Fair to poor
Cis-trans at ring	Good to poor
Optical isomers	Poor
Number of double bonds	Good
Conjugation of double bonds	Good to fair
Number of nonpolar substituents	Fair to poor
Number of polar substituents	Good
Polarity of the substituents	Good

Table III. Chromatographic Separability of Inorganic Elements in Relation to Variations in Properties

Kinds of Variations	Separability
Ionic charge	
Different elements	Good
One element (oxidation-reduction states)	Good
Ionic radii	
Different elements	Good to fair
Similar elements (rare earths)	Fair
Ionic mass	
Different elements	Good
One element (isotopes)	Poor

When molecules or ions differ but slightly in the parts or groups that are weakly attracted by solvent or absorbent, mixtures of the solute molecules are difficultly resolvable by means of chromatographic methods. This is true of large organic molecules which differ with respect to the number or the position of small nonpolar groups. It is characteristic of the optical antipodes in which identical groups are exposed to the absorptive phases. It is typical of the isotopes wherein the difference in size, mass, and charge of the atomic nucleus is small relative to the solvation of the ion and relative to the high polarity and binding force of the unshared electron.

What conditions must be found in order to facilitate the separation of optical isomers and of isotopes by chromatographic technique? Obviously a solvent and an absorbent that will exhibit preferential affinities for the components of the mixture are required. In the case of optical isomers, many selective systems occur in nature as indicated by the specificity of enzymes and by the selectivity of the metabolic systems of living organisms. Similarly, organic reactions and the exchange systems encountered in the photosynthetic apparatus of living cells appear to utilize one isotope of carbon in preference to another (*18*, *19*). Many plant and animal cells accumulate certain inorganic ions and exclude others of the same periodic group against tremendous concentration gradients. Perhaps if more were known about the mechanism of these selective processes, some of the partition mechanisms might be adapted to use in columns, or analogous partition mechanisms might be devised.

CHROMATOGRAPHY BY ADSORPTION

Chromatographic analysis based upon the use of surface-active adsorbents is the most selective procedure for resolution of a great variety of organic compounds. It has been applied to the detection of the smallest structural differences among organic molecules such as those involving molecular size, molecular or structural isomers, number of double bonds, conjugation of double bonds, position of double bonds, and number and position of various polar and nonpolar substituents (*47*, *62*, *69*, *98*, *142*). This adaptation of the chromatographic technique continues to yield significant results when utilized for the examination of a variety of natural products, especially those that are soluble in organic solvents. Selected recent examples are the investigations of the chloroplast carotenoids (*33*, *62*, *63*, *122*) and related compounds (*88*, *98*, *142*), of vitamin A contained in lipides (*23*) and prepared synthetically (*114*, *135*), of retinene and similar compounds of the visual-purple complex (*132*), and of various unsaturated fatty acids (*73*, *136*). The method has been utilized with vitamin B_{12b} (*97*), the abalone-shell pigment (*26*), a pterin-like pigment from the tubercle bacillus (*31*), chlorophyll from blackstrap molasses (*9*), and various common indicators (*60*). It has been employed with a paralytic poison that accumulates in shellfish (*115*), with a polymeric-homologous series of sugar acetates (*34*), with various amino acids (*124*), and with various inorganic ions (*110*, *111*).

The detection of small molecular differences by the use of surface-active adsorbents usually depends upon the specificity of the active surfaces. Thus far, this specificity has been discovered largely by chance. Now that the significance of the specificity is recognized, a systematic investigation of this property may yield results of great practical value. The suggestion by Pauling, that the presence of a solute during the preparation of an adsorptive agent may influence the adsorptive properties, points to one course of investigation. The relationship between the dimensions of a solute molecule and its ability to fit within the interplanar spacings of the adsorbent (*78*) points to another profitable course of research. In spite of many investigations of adsorption phenomena, a thorough analysis of the nature of the attractive forces between adsorbents and various parts of organic molecules (*69*, *120*) and between adsorbents and inorganic ions (*110*, *111*) remains a challenging problem (*110*, *111*, *129*).

CHROMATOGRAPHY BY PARTITION

The resolution of mixtures by partition between water bound in a stationary phase and an immiscible solvent as chloroform, toluene, or butanol continues to find wide application (*69*). The water may be fixed to a hydrophilic absorbent, as an aqueous gel, diatomaceous filter aid, starch (*117*), or filter paper (see section on paper chromatography). Examples of separations achieved by this method are the resolution of mixtures of ribonucleosides (*104*), of amino acids in a column of moist starch (*117*), of sugars and methylated sugars in a column of cellulose (*58*), of tropic and atropic acids in a column of silica gel (*50*), and of anthocyanidines in a column of silica gel containing phosphoric acid (*116*). Mixtures of various lower fatty acids have been resolved in columns of siliceous earth impregnated with methanol (*103*), with strong sulfuric acid (*94*), and with strong sulfuric acid plus phosphoric acid (*55*). Silica columns have also been employed for isolation of radioactive citric acid (*101*).

CHROMATOGRAPHY BY ION EXCHANGE

With few exceptions, ion exchange chromatography is most useful for the resolution of mixtures of ionizable or dissociable solutes. Through preparation of strongly acidic and strongly basic organic resins, the usefulness of ion exchangers has been extended to very weakly basic and acidic solutes. For the most part, the preparation of these organic ion exchangers has been in the hands of industrial organizations that have been interested in products of great stability, of high exchange rate, and of great exchange capacity. The exchangers that have been placed on the market represent an extremely small fraction of the great number that might be synthesized.

With the organic, resinous ion exchangers, the stability, permeability, exchange rate, and exchange capacity are related to two principal structural features, the degree of polymerization (usually called the degree of cross linkage) and the nature of the reactive groups. The degree of polymerization determines the permeability of the resin to solutes and to solvent so that it affects the absorption capacity and rate of exchange. This, in turn, affects the rate of migration of the solutes in columns as well as the distribution of the solutes in the absorption zones, especially in the diffuse trailing boundaries of the zones (*59*, *87*, *119*).

Thus far, only a few kinds of reactive groups have been incorporated in the organic resins. Most of these are sulfonic acids, carboxylic acids, and tertiary or quaternary amines (*87*). Although many investigations indicate that these groups react by ion exchange, recent studies of a basic resin (Duolite A-2) manufactured by the Chemical Process Company indicate that addition reactions with fatty acids as well as ion exchange may be involved with a single kind of reactive group (*107*).

Sources of commercial resins and many of the chromatographic applications in organic and inorganic fields have been reviewed by Nachod and his collaborators (*87*) and will not be repeated here. It is significant, however, that ion exchangers are finding extensive use in the concentration and purification of particular substances from various natural products as, for example, traces of radioactive elements in urine (*112*), a paralytic basic poison from shellfish that feed on the marine dinoflagellate *Gonyaulax* (*115*), the separation of various mononucleotides (*24, 25*), the estimation of riboflavin (*44*), and the separation of various acidic and basic amino acids (*87*).

After the extensive wartime separations of rare earths and fission products with ion exchangers (*59*), additional attention has been directed to the separation of zirconium and hafnium with acidic and with basic resins (*65, 123*). Radioactive isotopes of cerium and yttrium have also been utilized as an aid in the investigation of column variables such as rate of flow, exchange rate, and pH of the solution (*128*).

On a small scale, resins may serve for the preparation of potable water from sea water (*87*). For commercial installations, however, distillation with efficient heat exchangers seems to be more efficient (*46*).

Studies of the physical and chemical properties of the synthetic exchange resins and of the natural exchangers, such as alginic acid (*3*), are continuing to yield results of value in the field of chromatography (*51, 54, 66, 81*). In place of the acidic and basic groups, various oxidation and reduction groups may be combined in the resinous structure (*20, 130*). These modified resins then become specific reagents, analogous to the specific reagents of Feigl (*39*), for chromatographic investigation of a variety of solutes. In this way, the use of resins in chromatography may be made as widely applicable as the use of chemical reagents.

CHEMICHROMATOGRAPHY

Many combinations of chemical reagents and solvents have been utilized for the separation of various solutes by chromatographic methods as pointed out by Meinhard (*82*). This use of reagents promises much wider application in the future because specific reactants may be utilized individually in columns, or in successive layers in columns, or combined with other absorptive or reactive solids (*32, 57*).

From the author's own experience, an illustrative example is the separation of ferrous and ferric ions in a column of Celite moistened with a solution of urea plus ammonium acid phosphate. When washed with a solution of these reagents, the ferrous ions migrate rapidly away from the ferric phosphate which precipitates in the Celite. The ferrous ions are readily detected with *o*-phenanthroline, the ferric ions with *o*-phenanthroline plus hydroxylamine or with sulfide. In many respects, these results may be obtained more quickly and more conveniently in strips of filter paper impregnated with urea plus ammonium phosphate.

PAPER CHROMATOGRAPHY

In the past year or two there has been a tremendous increase in the number of reports concerning paper chromatography, the resolution of mixtures by differential absorption in strips (Figure 2) or in sheets of paper (Figure 3) (*28, 30, 69, 118*). This new interest stems from the ease with which many separations may be made, from the large number of solutes that may be detected simultaneously in a single mixture, from the minute quantities of solutes that may be separated, from the ease with which R values are determined, and from the convenience with which colorless solutes may be located in the paper after formation of the chromatogram.

For chromatographic separations in paper, only about 0.01 ml. of solution containing from a few micrograms to a milligram or two of solute may be absorbed. If a larger volume of dilute solution is necessary to provide sufficient solute, the solution should be concentrated before addition to the paper or as it is added to the paper (*131*). In paper chromatography, as in columnar chromatography, the initial width of the absorption zone should be small relative to the distance moved by the band (Figure 3) (*41, 119*).

Chromatograms may be formed in paper with water or with various organic liquids as solvent. Many of the organic liquids, such as butanol, furfuryl alcohol, collidine, lutidine, piperidine, and phenol are treated with water so that partition effects (Figure 4) predominate in the resolution of the solute mixtures.

Evaporation of solvent from solutions in paper exposed to air results in an increase of the R value; hence for quantitative results, the paper must be supported in a closed space saturated with the vapor of the solvent. Narrow paper strips may be placed in a stoppered tube (*108*), but large filter sheets, such as those required for two-way chromatograms, require a large cabinet, a large battery jar, or a glass aquarium tank (Figure 3). When the solvent is permitted to flow upward in the paper by capillarity, the paper sheets may be bent into a cylinder (*140*) or spiral (*77*) that will fit into a covered glass cylinder (*134*). When strongly absorbed solutes require extensive washing with fresh solvent, a pad of absorptive paper may be clamped to the end of a short sheet of paper (*84*) or a large portion of the paper sheet itself may be rolled up like a window curtain. For downward flow of solvent in paper, the liquid must be placed in an elevated trough (*4*) from which the paper is allowed to hang in a closed vessel.

Formation of a chromatogram in paper may require a few minutes when the solvent is permitted to penetrate only about 5 cm., but with a penetration of some 15 to 30 cm., about 10 to 24 hours may be necessary.

Paper chromatography has been modified for the separation of acidic, basic, and neutral amino acids by passage of an electrical current along a moist paper strip (*137*), and across a sheet of filter paper while solution was being drawn upward by capillarity (*53*). It has also been utilized for separation of inorganic ions by passage of current through a stack of moist filter paper disks, one of which contained the mixture to be resolved (*45*). Flow of solvent through the paper has been observed to yield small potentials that may affect the separation of adsorbed solutes (*109*).

Filter paper disks in the form of a column have also been utilized to increase the amount of solutes separable in paper (*85*). A thick pad of paper may likewise be substituted for a thin sheet of paper.

In paper chromatography, salts often separate into ions which may interfere with the isolation and identification of other substances (*29, 127*). From the reviewer's experience, ferric chloride yields hydrochloric acid which migrates away from the ferric ion, leaving ferric hydroxide precipitated in the paper. Likewise, sodium sulfide yields hydrogen sulfide which volatilizes, leaving sodium hydroxide in the paper. Impurities in the solvent (*36*) or in the paper, as copper salts (*90*) and hydrolyzable peptides (*141*), sometimes yield spurious results.

In view of the small amount of solutes separated in the paper chromatograms, extremely sensitive methods are required for the detection of the resolved compounds. Illustrative examples of the kinds of materials investigated by paper chromatography and the methods utilized for their detection are summarized herewith.

Radioactive products of photosynthesis were located by photography (*5, 18, 19*); nucleic acids were located by absorption of ultraviolet light determined photographically (*80*); penicillins (*61, 64, 74*) and streptomycins (*138*) were determined by bactericidal tests; ascorbic acid and related compounds were determined with 2,6-dichlorophenolindophenol (*79*); reducing and nonreducing sugars were determined with weakly alkaline permanganate (*91*) and with aniline phthalate (*92*); and amino acids were located with ninhydrin (*1, 7, 15, 68, 75, 89, 99, 102, 106, 139*) or with copper salts plus ninhydrin (*32*). Lysine has been detected with ferrocyanide (*2*) and glycine with *o*-phthalaldehyde (*93*). Other materials studied by paper chromatography were proteins and enzymes, located with benzidine, hemin, and peroxide (*43*); nucleotides of ribonucleic acid (*22*); iodoamino acids of the thyroid located by radioactivity (*127*); amino acids of frog larvae (*75*), of royal jelly (*102*), and of diphtheria toxin and toxoid (*139*); and a toxic substance formed by treatment of proteins with nitrogen trichloride (*6*). Still other substances investigated by adsorption in paper were 2,4-dinitrophenylhydrazones (*21*), dinitrophenyl derivatives of amino acids (*96*), decomposition products of riboflavin (*52*), pterins (*49*), epinephrine and norepinephrine (*48*), and various nucleic acids (*80*). Various amino compounds have been located with iodine (*12*).

A number of the chemical elements have been separated in paper where they are readily identified by the standard analytical reagents. Examples are the chlorine group of anions (*72*), heavy metal ions (*17*), aluminum and beryllium (*90*), various cations with *R* values (*100*), and numerous cations and complexes (*67, 70–72, 76, 110, 111*).

Optical scanning methods have been devised for the detection and for the quantitative estimation of the solutes separated in the paper (*15, 40–42, 86*). The shape and the area of the absorption zone also provide clues to the nature of the absorptive forces and to the amount of solute present (*13, 40*).

In spite of its limitations as a preparative method, paper chromatography shows promise of much wider application in the future. It is especially useful for investigation of substances that are readily soluble in water and alcohols but that are insoluble in the hydrocarbon solvents. In this respect, paper chromatography extends the usefulness of chromatographic techniques.

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PAPER CHROMATOGRAPHY

DORIS L. CLEGG
Washington Square College, New York University, New York, N. Y.

> **"A good technique sometimes renders more service to science than the elaboration of highly theoretical speculation."—Claude Bernard**

THE most recent major advance in the field of adsorption analysis is the substitution of filter paper for the column adsorbent (*81*). Originally the discovery of Schoenbein in 1861 (*176, 177*), while investigating the conditions of ozone formation under the influence of electrical discharges, he termed the phenomenon "capillary analysis" and predicted its future tremendous usefulness as an analytical tool.

Eighty years later, Gordon, Martin, and Synge (*81*) extended this technique to two-dimensional chromatography after a long period of quiescence in this field. Because their adaptation was based on the partition principle, the filter paper being the stationary phase which supports the static liquid phase (water in the cellulose) and the mobile liquid phase being the developer, this process was called "paper partition chromatography." However, even though the majority of workers employ this expression as an accurate designation of what happens, other investigators wonder if frequent absorption of solvent by the cellulose followed by elution does not occur during this process. Therefore "papyrography" (papyrus: paper, and grapho: write) was next suggested (*186*) as a more apt phrase; "papergram" (*3, 196*) instead of "paper chromatogram" designated the completed result.

The use of paper as the adsorbent is a particularly happy choice, inasmuch as amounts from 300 (*39*) to 5 micrograms (*160*) and even to 1 microgram (*105, 145*) have been analyzed quantitatively with an accuracy of $\pm 5\%$, thus serving as a pilot for later more extensive operations, or as an elegant conservation of a precious research compound. Paper is also very convenient, because it is easily stored in a small space, ready for instant use; can be mounted as permanent records (a spray of artist's "fixative" preserves colors against fading too rapidly); photographed wet, if drying causes change or loss in colors of unknowns (*148*) or interference due to increase in background intensity (*77, 148*); or photographed dry (*143, 145*) for preparation of slides or simply to store for future reference. Purity of substance or the presence of unsuspected constituents may be easily determined (*104*). It is also inexpensive; one 9-cm. circle will produce one complete separation using the Rutter technique (*173*) or six if the ascending or descending paper strip is employed; or 450 individual chromatograms per day can be accomplished using a series of concentric cylinders as described by Williams and Kirby (*207*). Paper chromatography is useful as a control of synthesis in industrial processes, because rapid qualitative analyses frequently can be made employing this simple but extremely effective method.

The extensive literature devoted to paper chromatography has been summarized in several review articles (*23, 32, 36, 37, 43, 60, 100, 135, 149, 157, 183, 195, 207, 208–211, 229*), perhaps the best and most recently available of which is that of Martin (*127*). This article is beautifully organized and extremely well written, covering the developments in both columns and paper to about January 1949. A collection of papers covering recent developments in the columns and paper is being published in the *Discussions of the Faraday Society* (*60*).

A great deal of ingenuity has been evinced in the various methods of handling the paper and in the form or shape of the starting sample: large sheets of paper (*81*) standing in 5-gallon crocks; strips of various sizes (*117, 144*); 13.5 × 1.8 × 1.0 cm. strips which are cut narrower at one end, so the wider part fits snugly in a test tube and the lower edge dips into the solvent (*171*); large 23 × 23 inch sheets stapled together to stand upright by their own sturdiness in a 20-gallon stainless steel tank (*224*); and the remarkably effective Rutter technique using circles or squares with a wicklike tail dipping into the solution (*173*).

Filter paper in various forms, has been utilized as a column adsorbent: as pulp (*28, 205*), as powdered cellulose (*94, 153*), as powdered Whatman ashless filter paper tablets (*94*), as mixtures of charcoal and pulp (*205*), as cellulose acetate (*19*), and as strips for descending chromatography, producing a "flowing filter paper chromatogram" by allowing the solvent to drip off the lower edge of the paper (*152, 154*). A recent development is the use of filter paper (400 to 450 sheets) with the sample absorbed by 20 to 25 sheets which are placed on top of the pile; the whole is clamped together between perforated stainless steel plates to form an excellent column (*138, 139*), and eluted as such. This is really an adaptation of the cotton fabric disk method of Lenoir (*112*). One investigator has utilized previously boiled string, cotton thread, or mercerized cotton thread (*117*), glass wool, and thin asbestos paper which form internally supported columns of very small cross section to accomplish descending and ascending separations on a definitely micro scale. A slight modification of the descending paper strip method is to attach a thick pad of cellulose at the foot of the strip to serve as a collector of the solvent; the operation is discontinued just before the fastest moving compound reaches that point (*137*).

The chromatogram is developed as a two-dimensional (*39, 201*), descending (*3, 105, 120, 138*) or ascending (*58, 207*) one-dimensional strip, or radially (*173*), depending on the substances examined and the preference of the investigator. All papers have a machine direction and must be cut perpendicular to it for best results. Some circular papers demonstrate this by developing as ellipses instead of perfect circles.

The advantages of columns have been combined with the elegance of paper chromatography by impregnating papers with various materials; the result then becomes the fixed phase. Filter paper has been impregnated with: (1) diluted vulcanized rubber latex for the separation of fatty acid esters on strips (*18*); (2) alumina for the separation of vitamin A (*47*), and then washed in ammonium hydroxide for the separation of vitamin A alcohol, esters of vitamin A, and retinene (*48*); (3) alumina for inorganic capillarity analysis (*95*); (4) an aqueous suspension of aluminum hydroxide to separate oxine compounds of aluminum, cadmium, zinc, magnesium, calcium, zirconium, and beryllium (*82*); (5) peptized alumina and formaldoxime hydrochloride for a ferro manganese spot test (*90*); (6) 20% phosphate buffer of pH 6.2 for the resolution of penicillin broths (*99*); (7) hydrated oxides of aluminum, silicon, or chromium used for inorganic chromatography (*71*); and (8) starch and fuller's earth for separation of vitamin A constituents (*48*). All these are incorporated into the pores of the filter paper fibers to complete the stable phase.

Various methods of evaluating the results have been used, depending on the compounds being investigated and the facilities of the laboratories concerned. Sometimes the completed chromatogram is sprayed with a solution to develop a color and the color is then compared photometrically with a known standard: flavenoid pigments (*206*); sugars sprayed with ammoniacal silver nitrate (*26*) or ammoniacal silver nitrate with resorcinol and naphthoresorcinol to identify reducing and nonreducing sugars (*73*); benzidine reagent to distinguish between sugars and urates (*91*); benzidine, ammoniacal silver nitrate, aniline-hydrogen oxalate, or phloroglucinol to identify reducing substances (*92*); reducing and nonreducing carbohydrates and derivatives analyzed by spraying with 1% aqueous potassium permanganate containing 2% sodium carbonate (*148*); or sugars are quantitatively weighed (*10*) and analyzed by hypoiodate oxidation (*85*).

Sugars (*70*) and amino acids (*156*) have been definitely identified by their optical rotation.

Radioactive sulfur (*190, 196*), iodine (*61, 188, 189*), and carbon (*11, 63*) are first separated from their various compounds on paper and then their positions are determined and quantitatively estimated by the use of Geiger-Müller counters, or by laying the paper chromatogram on a photographic plate, when a radioautograph results.

Visual examination revealed the position of the components of many mixtures after chromatography owing to their brilliant colors (ascorbic acid, *124*). Zones were detected by observing changes in the refractive index of the solution in contact with the particles of the adsorbent (*34*). Two glass windows are clamped between a U-shaped metal plate and the paper is placed under the thicker polished window. When observed at the proper angle and at a suitable distance, the boundaries of zones are easily seen.

Bioautographic study is another extremely useful tool for the quantitative determination of various separated components as in vitamin B_{12} factors (*46, 214*), vitamin B_6 (*217*), and alternate factors capable of supporting growth of *L. citrovorum* 8081 (*217*). Here, the paper is usually cut in strips, soaked in the solution being tested, and then pressed on an agar plate seeded with the selected organisms (*46, 214, 215, 217, 218*). The paper can also be cut into tiny squares and placed in a tube of bacterial culture (*227*).

X-ray and electron diffraction patterns (*164*) were used to identify various amino acids after they were leached out from the completed chromatogram (*33*).

The formation of a copper phosphate complex (*165*) permits identification of amino nitrogen by evaluating the copper in the filtrate. One to 25 micrograms of glycine and valine were determined by this method by decomposing the copper complex with diethyl dithiocarbamate, extracting the yellow color with amyl alcohol, and measuring the color against standards (*222*). α-Amino acids can be neatly determined by absence, because they form copper complexes which do not move with the solvent.

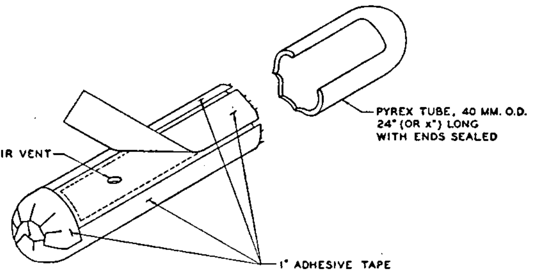
The paper is dusted with powdered basic copper carbonate as a streak along the future path of the first solvent (phenol). The mixture of acids is placed on this paper as usual and also on another paper without the copper compound, and then the two are chromatographed together as two-dimensional chromatograms, using as solvents phenol and then collidine-lutidine. One chromatogram shows spots for each component, but the paper containing the α-amino acid–copper complexes shows spots for all components except the α-amino acids, which remain at the bottom of the paper because they do not move with the solvent. Their positions are then found by difference by comparing with the standard chromatogram (*45*).

Another variation on the use of copper complexes of amino acids is to develop and dry the chromatogram as usual and then paint with freshly prepared 10% solution of aqueous ferrous ferrocyanide. The pink color of copper ferrocyanide betrays the position of the acid (lysine, *1*).

Amino acids are usually separated by two-dimensional chromatography and, after drying, the chromatogram is sprayed with ninhydrin solution, which produces various shades of blue or purple in a characteristic sequence of spots on the paper (*39*). This pattern is so definite that some investigators use the relative position on the paper to identify the components present (*119, 170*). The R_F value is the ratio between the distance traversed by the component and the distance traveled by the solvent.

Inorganic compounds can be separated by paper chromatography and then eluted, or the organic matter can be destroyed and the metallic content determined by use of the polarograph, spectrograph, or colorimeter (*3*).

Ultraviolet light is very successful in determining the position of many compounds which fluoresce: pterins (*80*), oxine compounds of aluminum, cadmium, zinc, magnesium, calcium, zirconium, and beryllium (*82*), amino acids and peptides (*158*), purines and pyrimidines (*204*), and nucleic acid derivatives (*125*). Viewed under ultraviolet light, the pale yellow glow of dinitrophenyl amino acids and peptides serves to identify the positions even if it is not a true fluorescence (*160*). Purine and pyrimidine derivatives do not fluoresce but quench fluorescence so that, if they are run using as solvent *n*-butyl alcohol which does fluoresce, dark patches appear where components are, against the bright background of the paper. Here the absence of this property is skillfully turned to account to analyze unknown substances. This same method is applied to various cations; the chromatogram is sprayed with selected reagents, such as morin, producing spots, some bright, and some dark through the quenching of fluorescence (*24, 162*). 8-Hydroxyquinoline (alcoholic) sprayed on a chromatogram of aluminum and beryllium produces fluorescence of each, so that their positions are easily established (*147*).



COURTESY, SCIENCE

Figure 1. Diagram of Glass Trough

Left. **Before cutting**
Right. **Completed channel**

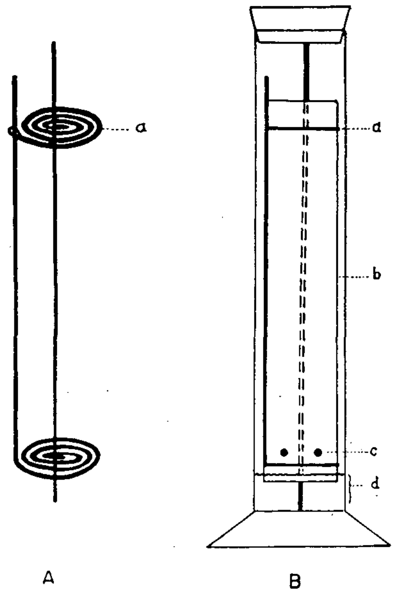
Acidic peptides are separated by ion exchange resins, followed by ionophoresis, which separates peptides according to their charges and molecular weights into groups having similar mobilities in an electrical field. Then these groups are examined by two-dimensional chromatography (*42*). Nineteen different dipeptides of aspartic and glutamic acids were identified in a hydrolyzate by this method (*42*). A special elution method was described (*49*).

Paper chromatograms are also quantitatively evaluated photoelectrically in the case of amino acids (*27, 76*), and metals (*136*). These results are plotted against the area and compared with similar plots of standard acids to get the concentration of the unknown (*16*), or measured with a planimeter, and the areas thus obtained are plotted against the concentration (*27, 66, 68*).

The transmittance of monochromatic light by mixtures of dyes has been automatically recorded during the entire process of development (*142–144*). Reflectance values of completed, dried chromatograms of mixtures of dyes were automatically recorded (*145*). Transmittance values are more accurate than reflectance values, because they follow Beer's law more closely (*212*). Reflectance records taken in this manner have limited value in accurately establishing the location of bands, but in a photometric sense are inferior to transmittance recordings.

Alternate methods of continuous scanning, including cathode ray tube scanning, have been discussed (*145*).

At present, all amino acids cannot be separated by one solvent, but an excellent beginning is accomplished by using two-dimensional technique and then cutting out the mixtures and further eluting in a one-dimensional chromatogram. The two-dimensional chromatogram is of widespread importance, because it is simple, easy, and extremely effective. One to 300 micrograms of the sample mixture are placed 2 to 3 cm. from one corner of a large sheet of filter paper. It is developed with a solvent, usually phenol for amino acids, taken out of the atmosphere and dried, rotated 90°, and inserted into a solvent of somewhat different properties, such as *s*-collidine or a collidine-lutidine mixture.



COURTESY, SCIENCE

Figure 2. Triangular Cross Section of Glass Trough

Amino acids occupy definite, characteristic positions on any two-dimensional chromatogram (*41*), and "maps" have been prepared showing the sequence of their positions. Inasmuch as, originally, they are colorless and, therefore, invisible, the paper is sprayed with ninhydrin to make a blue to purple spot depending on the amino acid concerned, which becomes visible on heating with steam. The ninhydrin is prepared as 0.1 to 1% solutions in water, butanol, or butanol–acetic acid. However, the procedure is not completely specific for amino acids, because any substance containing free amino and free carboxyl groups will produce the same result. The solvents used for amino acids are usually (1) phenol or butanol–acetic acid, or (2) collidine or collidine-lutidine. Sugars can be successfully chromatographed using the same two-dimensional technique and even the same solvents; however, benzidine or ammoniacal silver nitrate is used to produce the color. The choice of a developer or solvent is empirical because, as yet, there appears to be no accurate method of predicting how a substance will act with a given solvent.

It is reported that the rate of movement of solvent is about the same, whether ascending or descending methods are used. The exact ratio of change in width of paper to change in rate has not yet been definitely established. The paper has pores, which should not be too coarse, but fine and firm—the coarser the paper the faster the rate of flow. An increase in temperature also causes an increase in movement of solvent and, therefore, of the ions. pH seems to affect the separation both as to completeness of separation and sequence of components (*57*). The presence of other ions usually affects R_F values, but not in the case of penicillin and amino acids (*182*).

APPARATUS

A glass trough to hold the solvent, which was designed by Longenecker (*118*), is shown in Figure 1; it is easily and inexpensively made from chemical resistant glass with the aid of adhesive tape in strategic positions. For the detailed exposition of the technique, the original paper should be consulted.

This simple piece of equipment was quickly put to constant use, with the immediate result that it was modified for greater ease of construction as shown in Figure 2 (*181*). The main improvement is that the trough is now composed of sheet metal with a V-shaped cross section with welded end plates and coated with resistant enamel to eliminate breakage of the glass.

Another apparatus which enables one to chromatograph several paper strips simultaneously (*213*) is composed of a Petri dish.

Both halves face upward and are connected by fusing to a glass rod, which is wound with absorbent cotton to maintain the humidity. A wire guard is placed around the circumference of the upper trough to hold the paper strips away from the side of the trough over which they hang. This wire guard is made by bending a stainless steel wire to form a scalloped circle, which has sufficient spring tension to grip the sides of the Petri dish trough; no other means of attachment is necessary. The whole is placed in a large cylindrical glass jar, a humidifying chamber, and covered with an inverted crystallizing dish. The lower trough contains the aqueous solution which provides the necessary humid atmosphere, and the upper trough contains the organic solvent used for the development of the chromatogram. The paper strip, suitably folded to dip into the organic solvent, is held in place by a glass rod bent in the shape of an arc. Figure 3 shows the apparatus as used.

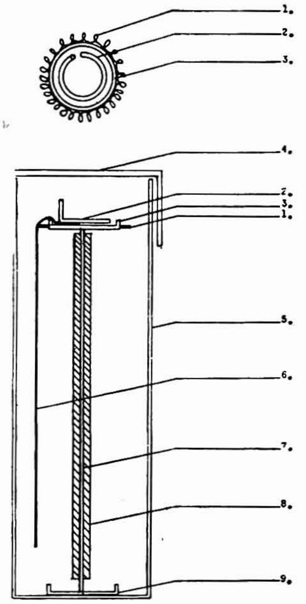
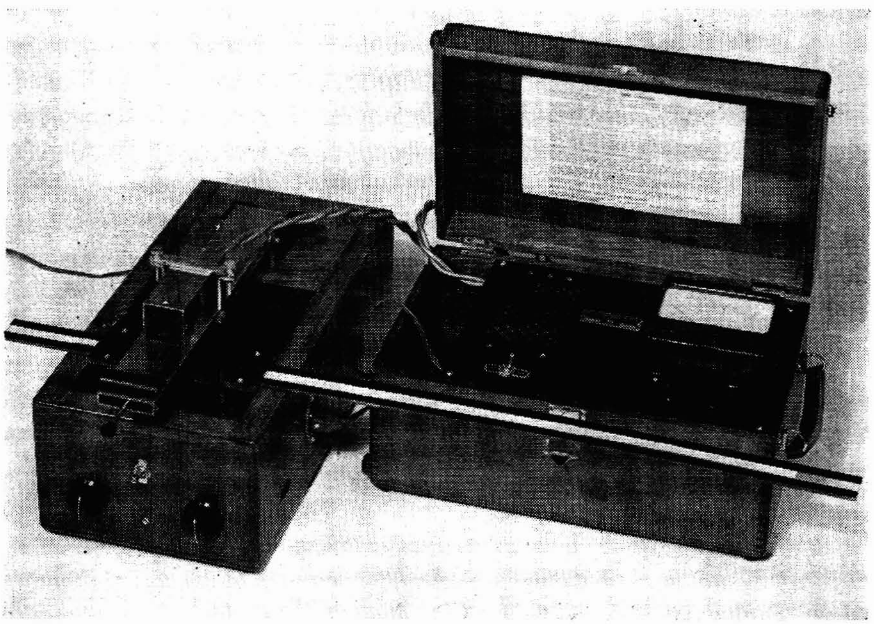


Figure 3. Apparatus for One-Dimensional Paper Partition Chromatography

1. **Stainless steel wire guard**
2. **Glass retaining rod**

3, 9. **Petri dish**

4. **Crystallizing dish**
5. **Cylindrical glass jar**
6. **Chromatogram strip**
7. **Glass rod**
8. **Absorbent cotton**



COURTESY, PHOTOVOLT CORPORATION

Figure 4. Photoelectric Photometer for Evaluating Paper Strip Chromatograms

Another glass trough (*4*) was made from chemical resistant glass tubing with only the assistance of a gas-air blowpipe and a 6-inch file. Mechanical details are given in the original paper.

An apparatus for scanning one-dimensional paper chromatograms was reported (*76*) in which the amino acid paper chromatogram, after development with ninhydrin, was passed between a light source and a phototube (Figure 4). R_F values and the quantity of unknown were determined by the use of transmittance values.

For investigators who are anxious to collect the effluent uniformly and gradually, there is a rotating drum method which permits 20 spots of effluent to be touched to the filter paper per revolution of the drum (*53*). Because about 10 revolutions elapse before the chromatogram is properly developed, some 200 spots will have been added at regular intervals from the graduated syringe.

Because it is both tedious and time-consuming to develop one chromatogram at a time, a setup has been reported which permits the simultaneous development of many chromatograms (*117*). The apparatus is shown in Figure 5.

A method (*98*) for treating paper with silicon esters renders the paper impermeable to water for 11 hours and also causes considerable resistance to alkaline solutions. This might well be of use in the formation of barriers and restricted channels on filter paper for chromatographic separations.

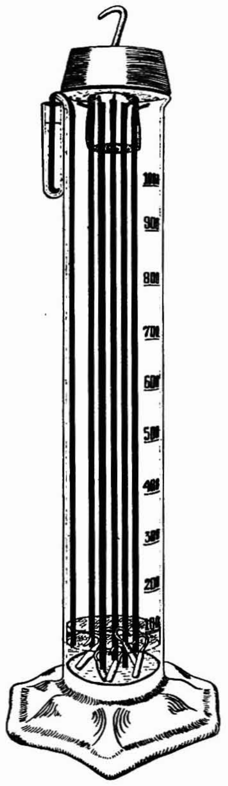
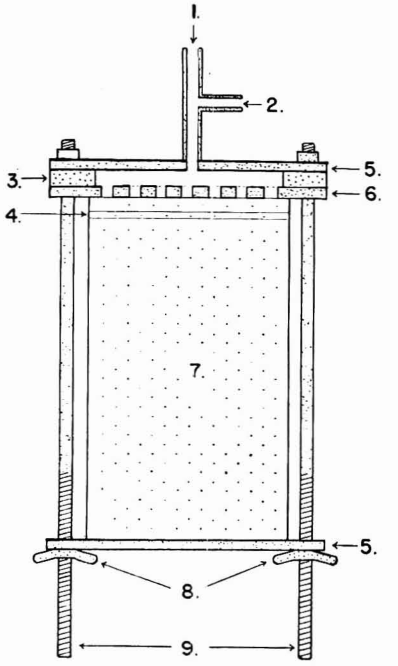


Figure 5. Apparatus for Ascending Filter-Paper Chromatograms

A new analytical technique for investigations of anions and cations by electrochromatography was reported in which adsorption is effected with the aid of e.m.f. (*107*) applied to a column. This might be a fruitful field of investigation for paper separations, especially of inorganic ions.

A special pipet, containing a platinum wire of a bore just fitting inside the capillary, permits the deposition of approximately 1 ml. per hour of the mixture being analyzed. It was reported that, because the solution evaporates from the paper strips at 60° C., the wet spots attain only 2- to 3-cm. diameter at any time, thus concentrating the unknown (*199*).

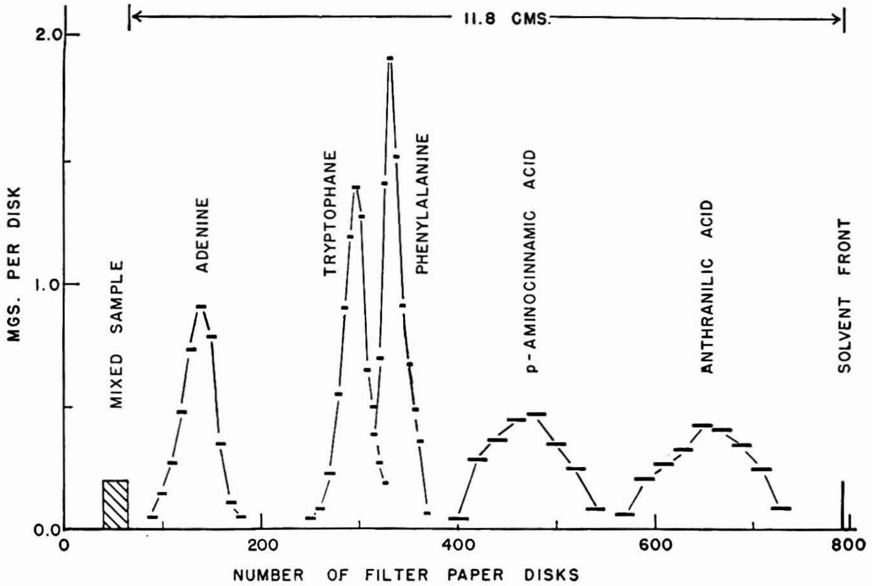
Another method of making the chromatography of amino acids quantitative consisted of plotting per cent transmittance on semilog paper against distance along the filter paper strips and measuring the areas under the curve with a planimeter. These transmittance values were obtained by mounting paper on a slide and passing it between an illuminated slit, 6 × 5 mm., and a phototube (*27*). A light filter with maximum transmission at 570 mμ was placed in front of the slit.



COURTESY, SCIENCE

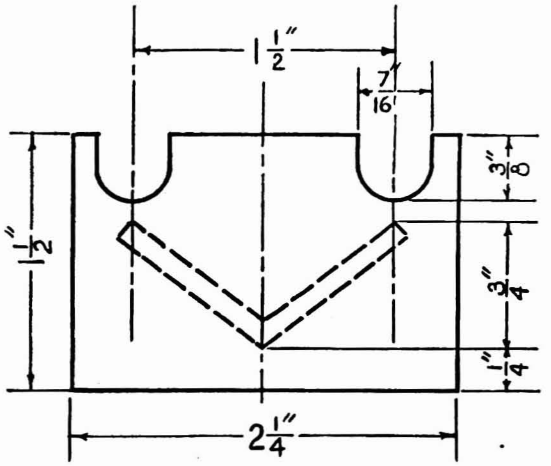
Figure 6. Diagram of Filter-Paper Pile Column

1. **Connection for rubber tube for filling siphon**
2. **Connection for siphon tube**
3. **Rubber gasket**
4. **Filter paper disks containing sample**
5. **Stainless steel plates**
6. **Perforated stainless steel plate**
7. **Filter paper disk pile**
8. **Wing nuts**
9. **Bolts at four corners of steel plates**



COURTESY, SCIENCE

Figure 7



COURTESY, SCIENCE

Figure 8

A method for producing a column from 400 to 450 filter paper disks (*139*) was designated by the authors as a "chromatopile," as shown in Figure 6.

Results of an experiment showing the separation of compounds of a known mixture are shown in Figure 7. The length of the horizontal bars used for experimental points indicates the number of paper disks extracted for each analytical sample.

A simple piece of equipment is composed of two long stainless steel rods which connect two stainless steel coils (*123*). The paper is held by the coils, as shown in Figure 8.

A simple accurate device for measuring R_F values was reported (*158*) by which a scale, divided into 100 parts, was printed on white elastic. One end of the elastic was fastened to the end of an aluminum strip and the other end to a bulldog clip. In use, the zero mark is placed at the origin line or spot and the 100 mark stretched out to coincide with the position reached by the solvent front. R_F values of the spots are then read off directly from the scale.

An apparatus was reported (*143, 145*) for automatically recording the transmittance of monochromatic light during the process of development of paper chromatograms of dye mixtures by capillary ascent. The dye mixture was deposited at the bottom of a restricted channel and the eluant was added at a point below. The monochromatic light was focused at a point above the unknown mixture. The transmittance was picked up by a photomultiplier tube, amplified, and then recorded automatically and continuously by a Brown recorder. The results were reproducible and accurate to ±1%.

An entirely different approach consisted of automatically and continuously scanning the paper strip during the entire process of development by the cathode ray scanning technique and presenting the results continuously on a cathode ray oscillograph (*145*).

An alternative method for evaluating a completed chromatogram has been devised in which the paper strip is wrapped around a drum and affixed in position with Scotch tape. The drum is rotated at uniform slow speed by a synchronous motor. A lamp, lens, and filter system project a narrow line of light on the strip and the reflected or transmitted light is picked up by a phototube connected to a Brown Electronik recorder. The instrument is shown in another article in this issue (*141*). An example of the information so obtained is shown in Figure 9.

In this case, a disk chromatogram of two dyes was developed by the Rutter technique (*173*). A diametral strip was cut from the finished chromatogram and mounted on the scanning drum. Reference lines were drawn in pencil: one through the origin or original sample site, and two others equidistant from it, well beyond the confines of the pattern.

In Figure 9 on either side of the central reference line, a band appears representing the location of the ring of blue dye. Beyond these, there is a pair representing the ring of red dye. Finally, there are the outer reference lines.

These results were obtained by reflectance. For an evaluation of concentration they are inferior to results obtained by transmittance, but they afford quantitative information on zone location, zone width, and symmetry. In this example, there is a notable lack of symmetry which would not be at all evident on simple inspection. If the maximal excursion of the solvent is marked with a pencil line before the solvent disappears by evaporation, these automatic records provide an accurate measure of R_F values. The scanning can be repeated over and over without attention from the operator.

THEORIES

A few theories have been advanced to explain the mechanism involved, the kinetics of paper chromatography, and methods of determining quantitatively the amounts of the various components. However, the time has not yet arrived when a new substance can be placed on a paper, a developer added, and the results accurately predicted. The first formula of any importance to paper chromatography originated with Flood (*69*), who related the zone radii to concentrations

$$r = f(\log c + A)$$

where r is zone radius, c is concentration, and A is a constant for the paper.

Hopf (*90*) tested this formula using filter paper treated with an adsorbent, and found that Flood's formula was applicable in most cases. He also derived an equation to denote the relation between two constituents, A and B, of a chromatogram. On development, the ring for A is bounded by radii r_1 and r_2, and the ring for B is bounded by radii r_3 and r_4. The initial solution is of known concentrations, a and b, respectively. Then $a = (r_1^2 - r_2^2)K_a$, and $b = (r_3^2 - r_4^2)K_b$, and values for K_a and K_b can be calculated. Also

$$a/b = \frac{(r_1^2 - r_2^2)Ka/b}{(r_3^2 - r_4^2)}$$

where $K_{a/b} = K_a/K_b$.

Glueckauf (*79*) determined complete isotherms within ±1% without a single concentration measurement.

LeRosen (*114*) derived a general term to standardize chromatographic adsorbents.

The partition of substances between aqueous and nonaqueous phases affects the distribution of substances around the center of the spot (usually elliptical) (*67*). The paper chromatogram was actually combined with microbiological assay methods for the quantitative analysis of the mixtures chromatographed. If s_1, s_2, u_1, and u_2 are known (s's) and unknown (u's) components, $s_1/s_2 = u_1/u_2 = k$. If corresponding areas of spots on the developed chromatogram are S_1, S_2, U_1, and U_2, then

$$\log \frac{u_1}{s_1} = \frac{(U_1 + U_2) - (S_1 + S_2)}{(U_1 - U_2) + (S_1 + S_2)} \log k$$

Fisher, Parsons, and Morrison (*68*) have shown experimentally that a linear relation holds between the area of the spot of test substance and the logarithm of the initial concentration. Brimley (*22*) has shown how this relationship can be derived by means of equations used in the theory of heat flow.

Martin and Synge derived an equation to express the relation between the complete separation of two solutes, 99.8 and 0.2%, and characteristics of the partition column (*131*) which was applied to the analysis of sugars (*97*)

$$\frac{A_2 + \alpha A_s}{A_L + \beta A_s} = \frac{r - 3\sqrt{r} + 2.25}{r + 3\sqrt{r} + 2.25}$$

where α and β are partition coefficients of the two solutes, A_L is the area of the cross section of the mobile phase, A_s is the area of cross section of the nonmobile phase, and r is the number of theoretical plates the first solute has passed.

Rutter (*174*) reported that the net resultant flow of solvent through the capillary channels of the paper used in chromatography is in a direction away from the point of feed and, therefore, sets up a streaming potential which he measured with a Cambridge pH meter. The potential gradients listed varied from 0.0+ to 10 mv. per cm. The rate of flow of liquid and the nature of the electrodes used in making the measurements appeared to affect the results.

Some inquiries into the kinetics of paper chromatographic development resulted in equations expressing the relation between R_F and the radii of successive zones (*144*).

$$R_F = \frac{r_2}{r_1} = \sqrt{\frac{b}{a}}$$

where r_2 is zone radius and r_1 is solvent radius.

In spite of the efforts of these investigators, the theoretical foundations of paper chromatography are in an extremely elementary state. In practice, the few generalizations that are the basis for predicting the optimum conditions for a new problem are well nigh useless. Even the selection of appropriate solvent mixtures is highly empirical and no guiding generalizations are known. We may hope that, in the near future, carefully controlled kinetic studies will clarify the role played by each of the variables, so that one may devise separations with greater certainty and with less recourse to empirical trials.

Figure 9. Automatic Record of Completed Chromatogram of Dye Mixture by Reflectance

APPLICATIONS

The applications of paper chromatography are primarily analytical and their greatest advantage lies in the minute amounts of material required for examination. The separation of the components of a mixture by diffusion through paper permits qualitative identification on the basis of the preferential rate of diffusion. Under carefully controlled and specified conditions, the R_F factor, which represents the ratio of the distance traversed by the substance to that traveled by the solvent, establishes identification. When a pair of components have almost identical R_F values, they may be resolved by further development with another solvent in a direction perpendicular to the first. This process is called "two-dimensional chromatography," and is being applied to clarify numerous analytical tangles.

INORGANIC APPLICATIONS

Schoenbein (*176*, *177*), studying the conditions of ozone formation under the influence of electrical discharges, observed selective adsorption of the components of a mixture by the different heights to which they rose when a strip of filter paper was dipped into the solution. This was the inception of filter paper chromatography, which he called "capillary analysis." Trey (*198*) first achieved separation of inorganic ions by this technique in 1898. After this time, many years elapsed before any further work was done on paper chromatography until Gordon, Martin, and Synge inaugurated two-dimensional chromatography for the separation and determination of amino acids. Because this technique was extremely effective and equally simple, it spread immediately throughout the scientific world, and soon many other substances besides amino acids were being separated, analyzed, isolated, and purified by this method.

Paper impregnated with aluminum hydroxide was used to separate inorganic ions by ion exchange (*69*, *95*). This method was further extended to the identification of several cations—aluminum, cadmium, zinc, magnesium, calcium, zirconium, and beryllium—as the 8-hydroxyquinoline derivative, the oxine. These oxines fluoresce under ultraviolet light, thus indicating their position for further identification (*82*). Chromic hydroxide caused even better separations than aluminum hydroxide (*82*). Various applications to metallurgy have been listed (*154*).

The use of radioactive tracers has greatly widened the field of applied paper chromatography, because much less than the 1 microgram required for color reactions is necessary for quantitative determination of such materials. Mixed chromatograms with nonradioactive material (known), in which colored and bioautographic spots correspond in position and shape, definitely identify the tiny tracer accurately. Substances containing radioactive isotopes were separated on one- (*189*) and two-dimensional (*11*, *61*, *63*, *188*) chromatograms and quantitatively estimated by the use of Geiger-Müller counters (*189*) or by laying the developed chromatograms on a photographic plate and producing a radioautograph (*61*, *64*, *161*, *171A*, *193*).

Rats were injected with I^{131} and killed after 24 hours, their thyroids were extracted, and the extracts were chromatographed. As a result, it was reported that diiodothyroxine and inorganic iodine have activity, but thyroxine has almost none (*61*); the large amount of I^{131} found in inorganic fractions suggested that an appreciable breakdown of organic iodine occurs during hydrolysis because the inorganic I^{131} that was initially present in the tissue had been removed by treatment with trichloroacetic acid (*189*); the rate data indicate that monoiodotyrosine is a precursor of diiodotyrosine (*188*).

C^{14} was administered to Chlorella as $C^{14}O_2$ to determine the significance of the form of compounds in photosynthesis (*63*). Plant respiration intermediates were studied using C^{14} as a label. The isolation and identification of C^{14}-labeled phosphoglyceric acid from the alga Scenedesmus which had photosynthesized for 5 seconds in $C^{14}O_2$ were reported (*11*).

Radioactive tracers and paper chromatography were used (*190*) to show that cystine and methionine are important sulfur compounds in alfalfa leaf proteins. Radiomethionine was administered orally to a rat, followed by the injection of benzene to study the benzene detoxification products (*196*). The paper chromatogram was read automatically by synchronizing a constant-speed motor pulling the paper strip slowly under the Geiger counter, with a tape recorder whose pen was motivated by counting impulses from the scaler.

Lederer (*108*) separated antimony from all the common metallic ions by acidifying the solution with hydrochloric acid, chromatographing the filter paper strip, developing with water until the chromatogram traveled 10 cm., and then adding hydrogen sulfide. This produced a characteristic orange patch of antimony sulfide close to the original spot, since antimony barely moves with the solvent. He also reported the separation of chloride, bromide, iodide, and thiocyanate ions, using as solvent butanol saturated with 1.5 *N* ammonium hydroxide, which keeps the halides in ionic form and prevents adsorption on the paper. R_F values were quoted (*111*).

Aluminum and beryllium mixtures were separated with 80% *n*-butyl alcohol–20% concentrated hydrochloric acid and determined by spraying with 8-hydroxyquinoline and examining under ultraviolet light, because their oxines fluoresce (*147*).

Lacourt *et al.* (*103*, *105*) quantitatively determined iron, titanium, and aluminum as chlorides and also cobalt and nickel as chlorides and nitrates. They reported that they could determine 10 micrograms of iron, titanium, aluminum, vanadium, nickel, and cobalt in methyl, ethyl, propyl, isopropyl, and isoamyl alcohol as impurities (*104*).

Burstall *et al.* (*28*) proposed methods for separating several cations with ketones. Gold, platinum, and palladium were separated from each other and from iridium or rhenium using methyl *n*-propyl ketone containing 30% concentrated hydrochloric acid. Chloride solutions of Group IIa (the copper and tin group) were separated with ethyl isopropyl ketone containing 10% concentrated hydrochloric acid. Group IIb compounds were resolved with dry ethyl acetate previously shaken with 2% hydrochloric acid. Nickel and cobalt in steel and minerals were separated and determined on cellulose pulp columns which had been activated by boiling with dilute nitric acid. Scandium was separated from many other metals, including the rare earths, with the salts as nitrates and using ether containing 12.5% nitric acid as the solvent by washing through the column of the cellulose pulp.

Calcium, barium, and strontium, as chlorides, were separated by an organic reagent, pyridine, containing 4% potassium thyocyanate as the solvent, and their positions were indicated by the use of sprays (*3*). Aluminum, gallium, indium, and zinc chlorides were separated with *n*-butyl alcohol containing hydrochloric acid (*3*). The same authors also separated and identified vanadium, mercury, cobalt, copper, iron, manganese, and nickel. Another method of separating the noble metals in aqueous solutions was reported (*109*); gold, platinum, palladium, copper, and silver were identified by their different R_F values and colors, and also their colors produced with hydrogen sulfide in an atmosphere of ammonia. In this case, an interesting separation occurred in the solvent of butanol saturated with *N* hydrochloric acid. Two liquid fronts appeared during the development, the first butanol and the second the aqueous phase, which was used as the reference for the R_F values. Mercury, silver, and lead chlorides were resolved with 5 *N* ammonium hydroxide and identified as sulfides by passing hydrogen sulfide vapor through the paper. Lederer (*109*) separated divalent copper and cadmium with 25% ethyl alcohol as the developer. Lead was separated from divalent copper, cadmium, and mercury and from bismuth by developing with 1 *N* aqueous hydrochloric acid, since lead does not travel with the liquid front (*110*).

Cations (24 of them) were separated using various solvents and then the spots were located by hydrogen sulfide vapor or by fluorescence, produced by spraying with a selected reagent, such as morin, drying, and then examining under ultraviolet light. Some cations fluoresce, giving bright spots, and some quench the fluorescence, yielding dark spots (*162*). Linstead (*116A*) stated that the rate of movement of any metal (R_F value) may be by the presence of other ions. Separations of 45 metals and anions, by successive changes of the extracting medium, were performed in his laboratory (not yet published). The metals were dissolved in a solvent, usually an acid, and the individual members determined by specific reagents or other conventional means.

SUGAR SEPARATIONS BY PAPER CHROMATOGRAPHY

Simple sugars were separated and detected by ammoniacal silver nitrate (*73*); and in two-component mixtures by using butanol–acetic acid–water as the solvent and analyzing by hypoiodate oxidation (*85*). Flood *et al.* standardized a procedure (*70*) and were unsuccessful in resolving *dl*-sugar mixtures by an optically active alcohol. Benzidine reagent was used to separate reducing sugars from urates (*91*). Sugars were also separated with collidine as the solvent (*153*); by spraying with a special reagent of aniline, phthalic acid, and water-saturated butanol, which produced red colors from aldopentoses and greens and browns from aldohexoses and uronic acids (*154*); using Ehrlich's aldehyde reagent for amino sugars (*2*); in aspenwood, spruce, and various coniferous pulp fibers by partial hydrolysis, usually resulting in mannose and glucose (*219*); and in several types of pine trees, where they were found to be arabinose and sometimes fructose (*220*).

The structures of methylated sugars were investigated (*9*), and it was shown that methyl irisin contains an appreciable fraction of trimethyl fructoses. These sugars were separated (*71*) on columns of powdered cellulose (*94*), and quantitatively identified by R_G values (*10*). They were separated like simple sugars and sprayed with ammoniacal silver nitrate to produce brown spots, which provided a method of rapid examination of hydrolyzed products obtained from small quantities of methyl polysaccharides (*26*). Reducing and nonreducing carbohydrate derivatives were separated and identified by spraying with 1% aqueous potassium permanganate containing 2% sodium carbonate to produce yellow zones on a purple background (*148*), and those in urine were identified by spraying with benzidine, ammoniacal silver nitrate, aniline–hydrogen oxalate, or phloroglucinol (*92*). Sugars present in urine were identified as arabinose, xylose, and fructose (*55*).

Methods of studying the sugars present in apple juice, egg white, and the fetal blood of sheep (*150*) and for the examination of blood group A specific substances from hog gastric mucin and the specific polysaccharides of *B. dysenteriae* (Shiga) (*151*) were described. The carbohydrate metabolism of microorganisms was studied using sucrose which was converted into dextran by *Leuconostoc mesenteroides* (Leister Institute Strain No. 3351) (*75*). The explanation offered was that during the course of the metabolism, large quantities of reducing sugars are formed and the glucose part of the sucrose is polymerized to dextran with simultaneous liberation of the fructose moiety. Sucrose was detected with resorcinol and estimated after preliminary hydrolysis with oxalic acid. Changes in the sugar content during the growth of the pea were studied by paper chromatography (*56*) and histochemical reagents were used to demonstrate pectin, starch, cellulose, and lignin development.

APPLICATIONS TO MEDICINE AND BIOLOGICAL CHEMISTRY

One of the greatest fields for the application of paper chromatography is in medicine and biological chemistry. The amino acid constituents of diphtheria toxin and toxoid hydrolyzates were determined (*223*) by two-dimensional chromatography, and it was found that tyrosine is present in toxin but not in the toxoid. Its usefulness in a routine method of preparing the toxin from the papain digest (*116*) was proved. An examination of the chemical nature of purified liver extract (*194*) showed the presence of peptide material, free amino acids, and riboflavin; the percentage of total activity was also studied (*178*).

Nucleic acids have been studied (*125, 202, 204*) and determined quantitatively both spectrophotometrically (*125*) and by ultraviolet absorption (*202*). Purines were detected by precipitation of mercury and conversion to a sulfide. Vitamin A was separated on alumina-treated filter paper and identified with the Carr-Price reagent (*47*). Substances related to ascorbic acid (vitamin C) were resolved on paper in several different solvents and then identified with 2,6-dichlorophenolindophenol, which produces a dark blue background around colorless spots (*126*). The various vitamin B's have attracted considerable attention (*46, 83, 179, 214, 215, 216*). The microbiological assay with paper chromatography has been used for the study of vitamin B_6 (*215*) with *Saccharomyces carlsbergensis* 4228 on agar and of vitamin B_{12} with *Lactobacillus leichmannii* 313 (*214*) and *Lactobacillus lactis* (Dorner) (*46*), with special attention to various related growth factors (*179, 214, 216*) and their effects on bacterial metabolism. The riboflavin decomposition products were shown by a paper chromatogram of irradiated samples of riboflavin and examined under ultraviolet light, under which they showed a yellow fluorescence (*83*).

Penicillin broth was chromatographed on paper impregnated with 20% phosphate buffer at pH 6.2 and quantitatively determined by the paper disk plate assay method. Eight penicillins were separated from *Penicillium chrysogenum* Q176 (*99*). Studies of two and three carbon compounds containing sulfur and sulfhydral groups showed no definite evidence that the ratio of penicillin formed is changed by their presence.

Paper chromatography of proteins and enzymes (*77, 138, 169, 192*) showed that enzymes are strongly adsorbed by paper at salt concentrations far below those required for precipitation, and that their movement is also influenced by the presence of other proteins, a form of displacement adsorption (*192*). Considerable enzyme resolution (*138*) and protein fractionation (*191*) can be attained by the use of a filter paper chromatopile by salting-out adsorption and adsorption displacement. pH affects R_F values because motion is almost nil to pH 5 and then it increases (*77*), and benzidine reagent brushed on the paper causes colors useful in identification. Some interesting microbiological assay investigations were performed using *Leuconostoc citrovorum* (*25, 217*) and some bioautograph methods, in which it was discovered that thymidine and possibly three or four other growth factors were present (*217*) in natural materials; that thymidine responds to an alkali-stable growth-promoting factor in concentrated liver extract and not to B_{12} (*25*); and that a possible functional relation exists between citrovorum factor and thymidine and pteroylglutamic acid—if used alone, growth was not seriously affected, but if used together, growth was greatly stimulated.

Paper chromatography has been applied to the clarification of many perplexing questions in the biological field: quantitative determination of ergothioneine in biological fluids (*225*); analysis of biological arylamines (*187*); detection of one or more hitherto unsuspected amino acids in the cholera Vibrio (*15*); and determination of the amino acid constituents of tissues and chromosomes (isolated from salivary gland cells) of *Drosophila virilis*, using acid hydrolyzed adults and larvae (*17*). Distribution of free amino acids in mouse epidermis, in various phases of growth (*170*), was decided by the relative position on the chromatogram and by comparison with a reference chromatogram, as a result of which it is believed that carcinoma can be sharply differentiated from the tissue of nonmalignant hyperplastic epidermis produced by the application of methyl cholanthrene. Estimation of some free amino acids in the tissues of the rat (*6*) demonstrated the presence of aspartic acid, glutamic acid, glycine, and alanine by spectrophotometry of the colors produced by ninhydrin on the completed chromatogram. The amino acid pattern in portal blood and jugular blood in dogs (*51*) was shown to consist of three types: those which rose and fell after the protein meal, those tending to rise slightly throughout the 5-hour period, and those that did not change during the experiment. The free amino acids in Haemolymph of *Calliphora erythrocephala* Meigen (*65*) were identified as alanine, tyrosine, proline, valine, isoleucine, histidine, phenyl alanine, glycine, leucine, lysine, serine, and aspartic acid.

A study of the chemistry of cacao fermentation (Forestero variety) (*74*) showed that (1) fresh bean pulp juice contained glucose, fructose, and sucrose; (2) citric acid was the only free acid in the pulp; (3) glutamic and aspartic acids and asparagine existed also in the pulp; and (4) tannins and other color constituents were extracted. Investigation of the amino acids in the tobacco mosaic virus indicated the absence of histidine, hydroxyproline, and methionine, but the presence of fifteen others (*87*). The free amino acids and peptides in frog embryos (*Rana pipiens*) (*115*) yielded only faint smudges of glutamic acid. The amino acids in Haemolymph of the dragonfly (*168*) nymph, *Aeschna cyanea*, showed the presence of serine, glycine, lysine, alanine, tyrosine, valine, leucine, and proline. The estimation of free amino acids in the tissue of rats showed the presence of glycine, alanine, and glutamic and aspartic acids (*5*).

The release of free and bound forms of biotin from protein (*20*) indicated that the solution liberated from liver by the crystal pepsin acted as a single compound, whereas that obtained from the countercurrent extraction of acid-hydrolyzed liver was resolved into several components. The toxic substance, gluten, was concentrated from Agenized flour (which causes fits in dogs) and resolved into glycine, serine, alanine, and glutamine (*29*). Peptides from insulin (*175*) on oxidation yielded four fractions of A and one of B, which are not all completely identified. Identification of Bence-Jones protein of multiple myelomatosis in amino acids by two-dimensional chromatography, using phenol and collidine as the solvents, indicated that no methionine was present, and all other common amino acids were demonstrated in the protein hydrolyzate except hydroxyproline (*50*).

APPLICATIONS TO AMINO ACID SEPARATIONS

The use of paper for the resolution of mixtures of amino acids was first proposed in 1944 (*81*) and its rapidity of separation, qualitative and quantitative results obtained, simplicity and reproducibility, all caused its immediate widespread adoption.

A spot of the mixture is placed about 2 cm. from one corner of a large sheet of paper and its lower edge is dipped into the solvent (usually phenol) and developed for 12 to 48 hours. The paper is then dried and rotated 90° and the edge is immersed in another solvent of somewhat different partition coefficient (collidine). This separates the amino acids efficiently, and, after spraying with ninhydrin in an atmosphere of steam, the blue to purple colors produced identify the various acids by their position on the paper. They can also be identified by cutting out the colored sections, eluting them, and titrating against 0.01 *N* barium hydroxide solution, using bromophenol blue as the indicator, or by their fluorescence in ultraviolet light.

Applications of this method are very numerous and results have been invaluable in elucidating structures, identifying compounds, separating impurities, and discovering new compounds.

The ninhydrin used as the spray to bring out the color of the amino acids is in 0.1 to 1.0% solutions in water, butanol, or butanol-acetic acid, and is reported to be sensitive to 0.1 to 25 grams of compounds detectable (*166*). A modified ninhydrin is suggested to be made of 2% ninhydrin solution in methyl Cellosolve water at pH 5 (citrate buffer), which contains stannous chloride as a reducing agent (*106, 140*). The latest innovation is the inclusion of 0.1% ninhydrin solution in the collidine or butanol-acetic acid used as the second developer; then only heat is necessary to produce the well known colors of the amino acids after the chromatogram is completely developed (*146*).

Some paper seems to have a peptide contaminant (*226*), which can be removed by washing the paper for 48 hours with water. The phenol used as the first solvent must be very pure and special directions are available as to the best method of purification (*54*), so as completely to eliminate all extraneous colors.

o-Phthalaldehyde is reported to be the specific reagent for the determination of glycine in paper chromatography (*155*). With acidified ninhydrin, no distortion in colors of R_F values of glycine, alanine, threonine, and tyrosine appears even when alkalinity is high, but with *s*-collidine the effect is reversed, the acid spot being near valine-leucine, and the alkali spot near glycine (*38*).

The formation of copper phosphate complexes is another method of separation and identification of amino acids (*165*) and ten such acids were investigated, with the decision that quantitative results can be obtained for those amino acids whose copper salts are soluble and can be applied to others which form insoluble copper salts. The distinctive test for α-amino acids depends on the fact that the copper complex, $CuCO_3.Cu(OH)_2$, forms a complex with α-amino acids whose $R_F = 0$, so that the other substances leave them behind at the bottom of the paper. Twenty amino acids were investigated (*45*). α-Amino acids and peptides can also be determined by their fluorescence under ultraviolet light (*158*). Amino acids can be separated on pulped filter paper and charcoal columns with good results (*205*). Iodine can be directly sublimed on a paper chromatogram or sprayed in alcoholic solution to reveal the position of choline and other *N*-methylalkanolamines (*21*). Furan derivatives (furfuryl alcohol and tetrahydrofurfuryl alcohol) containing 10 to 30% water produce good separations of amino acids on paper (*13*). It was reported that the inclusion of 0.5% urea, 8% pyridine, or 5% furoic acid as stabilizers sharpens the outlines of the spots and reduces their size.

There are many special applications in this field of one- and two-dimensional chromatography, such as identification of α-amino adipic acid (*14*); investigation of the action of nitrogen trichloride on proteins and attempts to isolate the toxic factor, which resulted in the conclusion that it probably is not a peptide (*12*); use of Pauly reagent to separate three monohydroxybenzoic acids from one another and from phenols (*58*); separation of isotopic derivatives of amino acids (*101*); and demonstration of the presence of thyroxine and diiodothyronine and triiodothyronine as ninhydrin reacting products from the hydrolyzates of thyroprotein (*88*). Chromatography of dinitrophenyl amino acids and peptides, which glow a pale yellow in a fluorescence which is visible but not a true fluorescence, showed that long nonpolar side chains cannot be resolved (*160*). Amino acid constituents of circulin were isolated on powdered cellulose and identified either by paper strip chromatography or by optical rotation (*156*).

Other applications include: determination of presence of esters and salts of fatty acids and amino acids in rancid cheese which cause staleness (*184*); and chromatography of amino acids belonging to homologous series, which fall on a smooth curve characteristic of that series and in the order of the molecular weight (*163*). Preliminary separation by ion exchange resins, followed by ionophoresis, separates the peptides according to charges and molecular weights into groups having similar mobilities in an electrical field; these groups were investigated by paper chromatography, and nineteen different dipeptides of aspartic and glutamic acid were identified in the hydrolyzate (*42*).

Norleucine was discovered to be absent in the spinal cord, contrary to the general opinion (*40*); steroids as hydrazones of Girard's Reagent T were separated and the dried chromatogram was dipped in iodoplatinate solution to remove the background, so the R_F values could be calculated (*228*); α-glutamyl peptides were synthesized and their purity was confirmed by paper chromatography (*113*); the copper phosphate-amino acid complex method was used to identify 1 to 25 micrograms of α-amino nitrogen (*222*). Amino acids were separated first by adsorption into dicarboxylic, basic, and neutral on anion and then on cation exchange resins and then the eluate was separated on paper strips, demonstrating the presence of the amino acids by ninhydrin, and quantitatively determining them by transmittance on an electronic densitometer (*16*). Studies were made of hydrolyzates of gramicidin (*185*); of protein fractions (flowing filter paper chromatographic technique) (*152*); of the amino acid composition of plant leaf protein fractions, showing that hydroxylysine was absent from leaf proteins (*122*), and identified the amino acids present (*121*); and of enzyme hydrolyzed proteins (*201*).

Quantitative determination of the components of many mixtures was reported on keto acids (*30*), purines and pyrimidines (*93, 203*), amino acids of salmine (*84*), *N*-substituted amino acids (*78*), purine and pyrimidine derivatives (*89*), lysine (*1*), creatine and creatinine (*132–134*), amino nitrogen (*223*), amino acid mixtures (*129*), and micro estimation of amino nitrogen (*221*).

Various experiments were followed by paper chromatography: amino acids, peptides, and compounds of tryptophan (*186*), action of alkalies on wool and deaminated wool (*35*), production of amino acids after a short exposure to $C^{14}O_2$ during photosynthesis (*180*), and a technique for demonstrating, by chromatography, the nitrogenous lipide constituents, sulfur-containing amino acids, reducing sugars (*31*), and relation of manganese and molybdenum to the free amino acid content of cauliflower (*86*).

There are several good review articles on amino acids (*128, 130, 200*), especially Martin's (*127*).

MISCELLANEOUS APPLICATIONS

α-Butyric acids were discovered in plant leaves (*52*). Various organic acids were resolved by using butanol–acetic acid mixtures and detected by spraying the dried chromatogram with an indicator (*119*). Potassium hydroxamate derivatives of organic acids were separated by suitable solvents (isobutyric acid and phenol) and the paper was then sprayed with dilute ferric chloride, which produced purple spots of the derivatives on a yellow background (*62*). This method is a very good means of identifying organic acids of one to eight carbon atoms. Many mixtures of nonvolatile organic acids were resolved and separated into their components (*120*). Ionization and adsorption of the acids by the paper were suppressed by swamping both the stationary and mobile solvent phases with a volatile acid. Formic acid produced well defined bands of the test acids (to travel down the paper sheet) and characteristic R_F values. Acids in carrot tissue were separated and identified by R_F value (*120*). Fatty acids, C_5 to C_{10}, were also separated (*167*). Dyes were investigated with cotton fabric disks (*112*), and in binary and ternary mixtures (*172*), absorbed on fibrous materials (*72*), and on ascending paper strips with automatic recording of the per cent transmission (*143, 145*).

Compounds of many other unusual substances were investigated by paper chromatography: pterins (*80*), identified by their characteristic fluorescence; dihydroionones (*96*); anthocyanines (*8*); hyoscine and hyoscyamine (*59*); flavins (*44*); flavonoid pigments in plant extracts (*206*); various lignins (*7*), which were identified by their fluorescence; and synthesis of amino acids by different types of bacteria (*102*).

SUMMARY

Paper chromatography has provided an extremely versatile tool, and in some types of work, notably biochemistry requiring analysis or separation on the microgram scale, it is almost indispensable. Many procedures are eminently practical for routine work. The considerable degree of empiricism that is still involved is a drawback in attacking a new problem. Further quantitative studies of a physicochemical nature, including kinetic studies, may enable one to evaluate the various factors involved in a more quantitative and predictable fashion.

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DISTILLATION

ARTHUR ROSE
The Pennsylvania State College, State College, Pa.

THE same general trends in analytical distillation have prevailed in 1949 as in recent years, and many of the statements made a year ago (*71*) still apply. Greatest activity has been shown in the design and testing of apparatus for the specialized conditions of vacuum and micro or semimicrodistillation. Additional details and applications continue to be presented on the precision analysis of liquid and gaseous hydrocarbon mixtures. The combination of distillation with other processes such as adsorption and extraction and identification of final fractions by such properties as infrared spectra are proving increasingly fruitful.

STILL HEADS

The most prolific single topic has been reflux dividers and condenser heads. It would appear that existing designs are all somewhat unsatisfactory, or that details and techniques are not properly worked out. At the same time, the problems seem simple enough from a superficial point of view, so that many workers are tempted to try working out a solution. Most of the new designs have been tested under a very limited range of conditions, and there are few objective comparisons. Tests of some devices in the author's laboratory indicate that actual reflux ratios are often far from what is intended. When proper weight is assigned to simplicity, reliability, ease of repair, and all-around utility, it appears that more attention might be given to careful techniques with the older types of heads and reflux dividers.

Doty (*17*) has described a vacuum-jacketed vapor-dividing reflux head that eliminates numerous sources of error. Hakala (*34*) reported total condensation and variable take-off without use of a stopcock. An electronic reflux ratio timer has been introduced by Fisher (*22*) and a device for low distillation velocities by Ynfiesta and Achon (*89*). Nord (*63*) has contributed to the problem of reflux division of streams containing water and an immiscible liquid. Diehl and Hart (*11*) devised a very low holdup head for vacuum columns. Their reflux divider, as well as that of Human and Mills (*40, 41*), features a long-stemmed glass valve. Goldsbarry and Martin (*30*) have patented a head with pressure-equalizing line to prevent backflow of condensate.

PACKING MATERIALS AND COLUMN EVALUATION

No entirely new packing materials have been described during the year. New test data deal chiefly with analytical columns of relatively large diameter. Cannon (*9*) has reported that 0.16 × 0.16 inch protruded nickel packing gives a height of equivalent theoretical plate of 1.4 to 1.5 inches in a 2-inch diameter column, and 1.8 to 1.9 inches in a 4-inch column. Fisher and Bowen (*21*) have compared McMahon packing (screen in the shape of a saddle) with several others, all in a 4-inch diameter column. Circular screen disks were tested as packing by John and Rehberg (*44*) in columns of 17-, 25.4-, and 50.8-mm. diameters. Dixon (*14*) tried out $^1/_{16}$- to $^1/_4$-inch metal gauze cylinders in columns of varied diameter and has reported as high as 84 theoretical plates per foot. A succeeding article (*15*) described the distillation of a multicomponent mixture with separation of materials having boiling point differences as small as 2.4° C. French (*24*) has given an application of gauze ring packing to distillation of coke oven by-products.

No further theoretical discussions have appeared on the complexities of evaluating column packing. Miller and Woodle (*55*) presented a paper at the September meeting of the American Chemical Society on the use of *n*-heptane–2,2,4-trimethylpentane as a test liquid. Evaluation at reduced pressure has been reported by two sets of authors with conflicting results. Berg and Popovac (*3*), using helix packing and *n*-octane–toluene as a test mixture, found no appreciable difference in number of theoretical plates over a range of pressures from 20 to 760 mm. Feldman, Myles, Wender, and Orchin (*19, 62*), using a variety of packings, worked with *n*-heptane–methylcyclohexane at atmospheric pressure and dodecane-cyclohexylcyclopentane at reduced pressures. Their results confirmed the prediction of Byron, Bowman, and Coull (*7*) of a broad maximum in fractionating efficiency somewhat below atmospheric pressure, with markedly lower efficiencies at lower pressures. More work is needed to clarify this situation.

SPINNER COLUMNS

Several spinner columns have been developed and some test data presented. This variety of column promises to be of real value in precise vacuum distillation. Donnell and Kennedy (*16*) have supplied additional details on a small concentric tube column, with stationary inner tube, which can separate 15-ml. samples into 0.5-ml. fractions for infrared analysis. Cruthirds, Jones, and Seyfried (*10*) reported H.E.T.P. values of 2 inches at atmospheric pressure and 4 inches at 0.1-mm. mercury pressure for a column 6.5 mm. in diameter with $^1/_4$-inch Chromel spinning band. Ten- to 200-ml. samples can be distilled in this apparatus. Larger rotating band columns have been described by Jost (*45*) with diameters of 12 and 50 mm., annular space of 1 to 2 and 1 mm., 800 and 6000 r.p.m., 100 and 350 ml. per hour throughput, and H.E.T.P.'s of 1.7 and 0.9 cm., respectively.

MICROAPPARATUS AND MOLECULAR STILLS

Bering (*4*) has reported a one-piece semimicro distilling apparatus with a spiral filled column for fractionating 1- to 4-ml. samples at 0.5 ml. per hour with a reflux ratio of 100 to 1. Lappin (*49*) devised a modified Cheronis distilling tube. A microfractionation still for samples between 0.1 and 0.4 ml. which supplies distillate fractions as small as 0.005 ml. has been patented by Gould (*31*). Babcock (*1*) described a similar apparatus designed for vacuum microfractionation and made by simple glassblowing from 5-mm. glass tubing, a test tube, and rubber stoppers. Several laboratory stills for molecular distillation have also been noted (*12, 29, 38, 74*), including one of the centrifugal variety by Biehler, Hickman, and Perry (*5*). Smittenberg (*78*) described the analysis of gaseous hydrocarbons on a micro scale by evaporation at low temperature and pressure.

ACCESSORIES

A number of distillation accessories or techniques have been reported. These include infrared lamps as a source of heat (*6, 68*), an automatic sample collector (*81*), a vacuum distillation sample collecting adapter without stopcocks (*76*), a simple relief valve for hazardous vacuum distillations (*58*), a recording differential refractometer (*90*), the use of ribbons of woven glass to prevent bumping (*61*), and other innovations (*26, 42, 46, 52, 60*).

ANALYSIS OF HYDROCARBON MIXTURES

Starr and Lane (*80*) presented an outstanding paper on accuracy and precision of analysis of light hydrocarbon mixtures, summarizing cooperative work by 70 companies, on 15 different mixtures of C_3 to C_5 hydrocarbons, and 8000 analyses by mass spectrometry, infrared and ultraviolet spectrophotometry, distillation, and chemical methods. The standard Rubber Reserve distillation procedures were used. Thirty-two laboratories reported 1716 values from distillation analysis. Compared with synthetic samples C_3 results averaged 0.6 mole % low, C_4 0.4 mole % high, iso-C_5 0.2 mole % high, and n-C_5 in close agreement. Precision was about ±0.4%. Individual laboratories were able to check their own analyses for a particular hydrocarbon within 0.3 mole %. Different laboratories checked within about 0.9 mole %. Distillation and mass spectrometry showed about the same precision and accuracy for total C_3, C_4, and C_5. Improvements in the distillation methods suggested later by Starr and co-workers (*79*) might result in even greater accuracy. A similar program on liquid hydrocarbon samples also involving low temperature fractionation is under way under the sponsorship of the Natural Gasoline Association of America. Miller (*54*) has issued a preliminary report. A series of hydrocarbon mixtures (C_3 to C_6) of known composition has been made available (*64*) for standardizing equipment and procedures.

The group at the National Bureau of Standards has continued the use of distillation as an important step in the analysis of a number of petroleum and other hydrocarbon mixtures (*18, 27, 28, 66*). Similar applications have been carried out by others (*13, 32, 50, 53, 69*). Several general articles on distillation analysis have also appeared (*20, 33, 72, 73, 84*). Some further development of A.S.T.M. and similar distillation methods has occurred (*35, 36, 48, 59, 70, 87*).

MISCELLANEOUS

Applications of analytical distillation to specific organic and inorganic problems are numerous (*2*, moisture in coal; *8*, water; *23*, lithium; *25, 77*, arsenic; *37*, furfural; *43, 88*, Kjeldahl; *47*, benzene; *51*, liquid air; *56*, acidity of milk; *57*, benzene; *65*, thiophene homologs; *67*, vitamin E; *75*, corn steep water; *82, 83*, fatty acids; *85*, nitrates; *86*, various elements).

Horsley has issued a 50-page supplement (*39*) to his original table of azeotropes and nonazeotropes.

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EXTRACTION

LYMAN C. CRAIG

The Rockefeller Institute for Medical Research, New York, N. Y.

THE present review is an attempt to continue along the lines initiated by last year's review. Although in the main the coverage is intended for papers dealing with analytical extractions which appeared during the past year, no claim is made for completeness. Almost any paper in the field of biochemistry or organic, physical, analytical, or inorganic chemistry potentially could have sections dealing with extraction which would be suitable for inclusion in the review. If reference to extraction has not been made in the title and the article has not appeared in the indexes of *Chemical Abstracts* as a paper on extraction, it will probably not be mentioned here.

EXTRACTION FOR REMOVAL PURPOSES

Relatively few papers dealing with simple extraction for removal purposes have appeared during the past year and these have been concerned mostly with apparatus. On the other hand, a larger number of papers have appeared in which simple stepwise countercurrent extraction has been employed to advantage for the separation of mixtures of closely related substances and as a criterion of purity for a given preparation. Unquestionably this latter type of extraction will receive the greatest attention for the next few years. The basis of this prediction is that apparatus have now been developed whereby thousands of quantitative extractions can be performed with but little labor. Moreover, the past year has seen several cases where extraction has resolved mixtures of substances whose physical and chemical properties have made them unsuitable for fractionation by other known procedures.

The first consideration in attempting to use extraction for a given purpose is the choice of a suitable solvent. A favorable solvent greatly simplifies the procedure by making complicated apparatus unnecessary because a few simple extractions can then accomplish the purpose. The properties of solvents can be modified greatly by addition of the proper solutes. An excellent example of this is provided by the interesting paper of Smith and Page (*35*). The possibility of the use of simple extraction for removing strong acids such as hydrochloric or sulfuric from aqueous solution would not occur to most chemists. Yet when the solvent is a chloroform solution of a long-chain tertiary amine, such as dioctylmethylamine, chloride or sulfate ion can be removed to the extent of 98% by a single extraction provided strongly basic ions such as sodium or potassium are absent. Indeed, the method should prove to be the one of choice for removing chloride or sulfate ions from protein or peptide hydrolyzates.

The unique distribution is based on the fact that the amine forms a salt which is preferentially soluble in chloroform and gives a water-chloroform partition ratio which greatly favors the organic phase. Not all tertiary amines are able to do this. Thus triamylamine did not give the effect but trioctylamine served the purpose. Alkaloid chemists have long known that the hydrochlorides of certain aromatic amines were more soluble in chloroform than in water.

Streptomycin, a basic sugar derivative and a strongly hydrophylic substance, can be made to partition preferentially in favor of an organic phase by the use of a solution of one of the anionic detergents. These agents have been called carriers by O'Keeffe, Dolliver, and Stiller (*27*). Among the agents tested and found to be carriers were fatty acids, alkylsulfonic acids, alkylsulfuric acids, and arylsulfonic acids. The pH of the aqueous layer was found to be an important factor in the numerical value of the partition ratio.

The same group of workers have also been able to partition heparin (*28*) by using laurylamine as the complexing agent. Heparin is a complex polysulfuric ester of a polysaccharide of high molecular weight. One cannot help but wonder if certain proteins might not be partitioned with the appropriate solvents.

Weizmann, Bergmann, Chandley, Steiner, Sulzbacher, and Zimkin (*43*) have made a study of the selective extraction of ethylene chlorhydrin. They found a distinct dependence of the selective solvent power on the molecular size of the solvent. The effect could not be ascribed to a definite compound formation such as a hemiacetal when ketones or aldehydes were the solvent but represented a more complex balance.

Derivatives of furan were found useful for removing fatty acids from aqueous solution (*15*).

A number of papers on the use of extraction in the inorganic field have appeared recently. The method has been applied to the separation of certain compounds of iron (*20, 44*), antimony (*9*), rhenium (*41*), gold (*23*), uranium (*26*), zirconium and hafnium (*4, 18*), cerium (*42*), nickel and cobalt (*11*), and thorium (*33*). Reasoning on the basis of the progress which has been made in the field of organic chemistry in the separation of closely related homologs or isomers, one can predict that much will be done by suitable countercurrent extraction and with the proper understanding of solvents. From the data in the literature, a problem would appear to be the working out of systems which give reasonably linear partition isotherms. It is surprising that a more serious attempt has not already been made along this line.

The correct evaluation of a continuous extraction process is not always easy and frequently is oversimplified by the use of certain assumptions which in actual practice do not hold. However, when the partition ratio remains nearly constant over a wide range of concentration, evaluation in terms of an analogous ideal discontinuous process is not too difficult. But in practice often the partition ratio changes so that extraction is favored as the solute is progressively extracted and the solution becomes more dilute. The opposite also may occur. In either case, calculations become involved. Bewick, Currah, and Beamish (*2*) proposed a method for the rapid evaluation of continuous extraction processes which includes a new value, the half extraction volume. They described an apparatus for carrying out comparative extractions and for getting the data from which the new value may be derived graphically.

Tsai and Fu (*40*) have extended the mathematics and approach required for the analysis by simple extraction of mixtures of the lower fatty acids.

Apparatus. Hsiao (*17*) and Lash (*21*) have described microextractors which use a cold finger type of condenser. Hsiao found his design to be excellent for the estimation of the fat content in biological materials and to be much more rapid than the Soxhlet extractor. Kolb (*19*) encountered difficulty in applying the extractor of Hsiao to pulverized mouse muscle. He described a stepwise procedure and an apparatus which overcame the difficulties.

When very small amounts of material are to be extracted, it is customary to use a small standard extractor. Even so, a much larger volume of solvent relative to the material to be extracted is necessary. Certain disadvantages such as introduction of impurities and promotion of transformation often accompany the use of too much solvent unless special precautions are taken. Stern and Kirk (*37*) described an efficient microextractor which not only is suitable for extracting small amounts of substance but also employs only a small volume of the extracting liquid. It is designed so that the ensuing operations such as evaporation are most convenient.

Chute and Wright (*3*) have developed a small laboratory continuous extractor which is designed to operate under a pressure of one or more atmospheres. Higher temperatures often contribute to more efficient extraction if the solute has sufficient solubility.

Nolan (*25*) gave the details for a continuous multiple extraction apparatus in which any number of analytical extractions up to 54 could be made simultaneously. It could be used for liquid-liquid extraction as well as for the extraction of solids.

Micaelli and Desnuelle (*24*) found a stepwise extraction of oil cakes to be more rapid and complete than a Soxhlet extraction. They used a separatory funnel with a sintered filter sealed into the funnel so that the extract could be withdrawn from the solid by suction.

EXTRACTION FOR FRACTIONATION PURPOSES

The success of extraction for fractionation purposes depends on a favorable state of affairs with regard to a number of factors which may be considered in three groups as follows: (1) mechanical considerations (apparatus); (2) availability of suitable systems and the required specificity or selectivity; (3) analytical and isolation procedures.

All three bear a certain relationship or dependence on each other. Thus, if an unlimited number of quantitative extractions or their equivalent were easily attainable, the second consideration would be minimized, as a highly selective system would not be required to achieve separations. Conversely, if a sufficiently high selectivity can be found, the mechanical aspects are greatly simplified since only a few stages are required for separations and the apparatus can be very simple. The analytical difficulties depend on the systems chosen as well as on the number of estimations which may be involved. Where incomplete separations are to be treated, both general and highly selective analytical methods are desirable. During the past year progress has been made in each of the three groups. Two of these will be treated separately. The third would include a review of the progress made in spectrophotometers, balances, and quantitative color reactions and is too broad a subject to be covered here.

Apparatus. Raymond (*32*) described a mechanically shaken countercurrent distribution apparatus for smaller numbers of transfers which employed especially designed separatory funnels. O'Keeffe, Dolliver, and Stiller (*27*) gave a useful separatory funnel procedure for larger volumes and for continuous introduction of solute. In this procedure a mixture is resolved into two fractions on a single passage through the series of funnels.

Craig and Post (*8*) gave a description of several types of countercurrent distribution apparatus. The details of construction of a 54-tube steel apparatus with glass plates on each end and instructions for regrinding the surfaces were given. The 54-tube apparatus can be operated so that it is analogous to a continuous column with 54 theoretical stages in it. Several methods of operating the apparatus have proved useful for different separations. These are the "fundamental" procedure, the "single withdrawal" procedure, and the "alternate withdrawal" procedure.

An all-glass type of distribution apparatus made with individual interlocking units was only briefly described (*8*) since it had not been extensively tested. An extraction train containing 108 such units was subsequently built. This was immediately successful in separating polypeptides of bacterial origin (*6*). However, experience gained in this work soon suggested improvements. The present stage of development in the author's laboratory includes a train containing 220 units of improved design. It is fully automatic with electric motors and time clocks attached. If the single withdrawal technique is desired, provision is made for this by use of an automatic filling device and by integration of the Stein and Moore chromatographic fraction collector (*36*) at the effluent end of the train. Thus the apparatus can be operated in a manner entirely analogous to chromatography. The advantages of such equipment are obvious and include the possibility of considerable expansion in regard to both the number of units and the size or capacity of each unit in the train.

Systems. Although one can gain a fair idea from the study of individual partition ratios and partition isotherms whether or not the separation of a given mixture is possible, actual resolution of the mixture by countercurrent distribution is much more convincing and at the same time gives more pertinent information. Therefore the subject will be covered in this review by referring to actual separations made with the countercurrent distribution technique.

Countercurrent distribution has been extensively used in separating and characterizing the streptomyces antibiotics (*27, 29, 31, 38, 39*) following the discovery of certain solutes to promote the desired partition as mentioned. Fortunately from the standpoint of separation, considerable specificity or selectivity appears to have been attained despite the complex equilibrium involved.

Titus and Fried (*39*) made an interesting observation in regard to tautomerization. Theoretically tautomerization should give a distribution curve wider than the theoretical for a single substance, if the time of establishing equilibrium between the tautomers is greater than the time between transfers in the machine and provided the pure tautomers have different partition ratios in the systems employed. Since determination of purity with the distribution technique involves an experimental curve which coincides with the normal curve of error, one must consider the possibility of tautomerization with certain curves which consistently come too broad.

Titus and Fried were able to show that streptomycin can exist in several forms involving a change of structure connected with the aldehyde group in the streptose portion of the molecule. The only method used for detecting the tautomers was their distribution curves. Yet clear evidence was presented and one of the readily interconvertible forms was isolated in a high state of purity by use of the proper system and temperature.

O'Keeffe, Russo-Alesi, Dolliver, and Stiller (*28*) studied the distribution of heparin by using laurylamine as the carrier. Two fractions were completely separated from each other. Both had heparin activity. With the start made on this type of substance, the question as to how far such an approach can be carried is foremost.

Naturally occurring compounds of phosphorus such as the phosphatides have long been difficult to fractionate and characterize. Promise in the use of countercurrent distribution for this purpose is given by the work of Scholfield, Dutton, Tanner, and Cowan (*34*) on so-called soybean lecithin. Although the work reported gave only a preliminary idea of the complexity of the mixture, it was sufficient to cause considerable revision of the views previously held. Another example dealing with a compound of phosphorus is the purification of cozymase, the diphosphopyridine nucleotide, by Hogeboom and Barry (*16*). In this work a number of experimental difficulties were skillfully overcome.

Although the lack of a method for fractionating and characterizing naturally occurring peptides has prevented the type of study the structural chemist prefers to do, nonetheless the importance of the field to biochemistry is well recognized. There are now available rapid methods (chromatography) for determining quantitatively and qualitatively the amino acid content in hydrolyzates but only laborious methods for isolating the amino acids in more than micro amounts and no suitable methods for isolating peptides from partial hydrolyzates. It would seem reasonable, if not certain, that polypeptides occur widely in nature even if few have been isolated in the pure state.

A degree of cautious hope appears to be warranted from the results of a number of papers dealing with the use of countercurrent distribution together with chromatography. Gorley (*14*) purified the polypeptide antibiotic, bacitracin, by countercurrent distribution. Craig, Gregory, and Barry (*6*) applied many more transfers to the purification and showed that it is perfectly possible to hydrolyze such a polypeptide and isolate the component amino acids in an analytically pure form by extraction. The amounts isolated are sufficiently large so that unequivocal identification may be made by the classical procedures.

Craig, Gregory, and Barry (*5*) were able to fractionate gramicidin, tyrocidine, and gramicidin-s each into families of polypeptides. The individual members of a family differed slightly by their amino acid components, but in some cases only by the stoichiometric proportions of the amino acids.

A beginning has been made in the application of extraction for the proof of purity in the polymyxin antibiotics (*1*).

Livermore and du Vigneaud (*22*) have purified the oxytocic hormone from the posterior lobe of the pituitary by countercurrent distribution and have given evidence for the purity of their preparation. The substance is quite unstable and therefore extraction would appear to be the most promising approach. Perhaps this work represents a beginning toward a better understanding of such hormones.

Woolley (*45*) isolated crystalline yellow peptides from the partial hydrolysis of insulin which had been reacted with dinitrofluorobenzene. This approach to unraveling the structure of proteins should become more promising with the feasibility of very high numbers of transfers.

The separation of the individual phenolic substances present in coal tar is a problem which has always been difficult. Golumbic, Orchin, and Weller (*13*) and Golumbic (*12*) have taken up the study of the separation of the complex mixture of phenols produced during the liquid phase hydrogenation of coal. Their results show that the technique is ideally suited, in many respects, to the problem. Phenols have characteristic absorption spectra in the ultraviolet, a point which makes the analytical work easy and which contributes greatly to the specificity. The type of data these authors give is the type needed for all classes of organic substances.

Reasoning on the basis of the experience with the quinoline synthetic antimalarials (*7*), a countercurrent distribution should be ideal for separating the individual members of the closely related alkaloids which exist so commonly in nature. Fried, White, and Wintersteiner (*10*) readily separated two new veratrine alkaloids by this technique from the amorphous residue which remained after the known alkaloids had been separated by the older published methods.

A very interesting study in the separation of the azulene hydrocarbons has been made by Plattner, Heilbronner, and Weber (*30*). Satisfactory partition ratios were obtained by using a system with a hydrocarbon such as petroleum ether or toluene for the one phase and a strong mineral acid such as sulfuric or phosphoric acid for the other. The strength of the mineral acid controlled the value of the partition ratio. Linear partition isotherms could be obtained at lower concentrations and equilibrium was established in less than a minute as has been found with the different classes of substances studied elsewhere. Data were given with the isomeric methyl azulenes to show that separation would be easy particularly with the equipment now available in this country. Beta values of the order of 2 or more were obtained. Shifting the phase pair, for instance, from petrolic ether–sulfuric acid to toluene–sulfuric acid caused a considerable shift in the β value for a given pair of isomers.

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ION EXCHANGE

ROBERT KUNIN
Resinous Products Division, Rohm & Haas Company, Philadelphia, Pa.

IN LAST year's review (*14*) of the application of ion exchange techniques to analytical chemistry, the basic principles of ion exchange and the general nature of ion exchange materials were reviewed, in addition to the analytical applications. During the past year, considerable progress has been made in the use of ion exchange as an analytical technique. In particular, ion exchange materials have proved themselves to be useful both in chromatography and in conventional analytical methods. In the latter case, the removal of interfering elements has been simplified in several conventional analytical procedures.

LITERATURE REVIEWS

In a chapter of a recent book on ion exchange, Rieman (*18*) has reviewed and classified the analytical applications of ion exchange. Its use in the laboratory, in particular, the chromatographic techniques, has been reviewed by Tompkins (*27, 28*). The usefulness of ion exchange in rare earth and radioisotope chemistry has been discussed critically by Cohn, Parker, and Tompkins (*6*) and Steacie and Cambron (*23*).

ION EXCHANGE CHROMATOGRAPHY

The separation of closely related ionic species by chromatographic techniques utilizing an ion exchange resin adsorbent has been applied successfully to several interesting inorganic and organic mixtures. Utilizing radioactive tracers, Tompkins (*26*) has shown that radium and barium can be fractionated in a manner similar to the rare earth separations. The citrate complexing technique has been used to develop into bands the constituents previously adsorbed on a sulfonic acid cation exchanger. Similarly, Street and Seaborg (*24*) have separated the closely related ionic pair, hafnium-zirconium. Kraus and Moore (*12, 13*) have employed a most interesting technique for the separation of the pairs, zirconium-hafnium and columbium-tantalum, utilizing anionic complexes of these ions. Oxalate and fluoride complexes of zirconium and hafnium were chromatographically separated on an anion exchanger of the strong base type. The complete separation of columbium and tantalum as the CbF_5^{--} and TaF_5^{--} ions was accomplished on the same resin, utilizing identical chromatographic techniques.

The separation of the ribose nucleosides, purine, and pyrimidine fragments of yeast nucleic acid has been accomplished in a most spectacular manner by Cohn (*3, 5*) using both anion and cation exchange resins. Utilizing a sulfonic acid cation exchanger, Cohn found it possible to separate completely the purine and pyrimidine bases, uracil, cytosine, guanine, and adenine. In order to separate the nonionized bases, thymine and uracil, as well as the other bases, Cohn employed a strong base anion exchanger as the adsorbent. The separation of the monoribosenucleic acids (uridylic, guanylic, cytidylic, and adenylic acids) has been accomplished by Cohn on a sulfonic acid exchanger. Because the acids exhibit but slight basicity, their elution from the column was effected with a weak acid. The method perfected by Cohn (*4*) is suitable for most laboratories, as the chromatographic development can be readily followed and traced by means of spectrophotometric absorption techniques in the ultraviolet (260 to 265 mμ) region.

Refinements in the ion exchange separation of amino acids have been made by Rauen and Felix (*21*) and by Partridge (*20*), the latter utilizing the Tiselius displacement technique.

REMOVAL OF INTERFERING ELEMENTS

Of considerable interest has been the utilization of ion exchange methods for removing interfering constituents. In many conventional methods of analysis, anions interfere in the analyses of several cations. Inasmuch as oppositely charged ions can be separated by means of ion exchange, these interfering ions can be removed readily. Sodium cannot be determined as either the zinc or magnesium uranyl acetate in the presence of phosphates, molybdates, and other anions that are capable of forming insoluble precipitates with the uranyl acetate reagent. Klement and Dmytruk (*11*) recommend the removal of phosphates by means of an anion exchange resin prior to the precipitation of the sodium. Linqvist (*16*) suggests the adsorption of sodium on a cation exchanger in order to separate the interfering elements. However, this latter method requires the additional acid elution step prior to the sodium precipitation.

The interference of copper, iron, and other cations in the iodometric determination of arsenic has been eliminated by the utilization of a sulfonic acid exchanger in a procedure devised by Odencrantz and Rieman (*19*). The procedure involves the solution of the sample, oxidation to the quinquevalent state, evaporation, re-solution, and passage through the cation exchanger in the hydrogen form. The arsenic is then determined iodometrically, using standard thiosulfate for the titration of the liberated iodine. This method eliminates the distillation step recommended by the Association of Official Agricultural Chemists. This procedure is analogous to that developed by Helrich and Rieman (*8*) for the determination of phosphorus pentoxide in apatite.

In the determination of copper, iron, aluminum, calcium, and magnesium, polybasic carboxylic acids such as oxalic and tartaric acids interfere because of their complexing and sequestering activity. Their removal usually requires a lengthy oxidation with nitric acid or aqua regia. In order to simplify the removal of these interfering anions, Klement and Dmytruk (*10*) have suggested the use of an anion exchanger in the chloride form. Sulfate trace impurities present in gelatin used in the nephelometric analysis of sulfates have been removed by Honda (*9*) with the aid of an anion exchange resin. It is probable that the gelatin, starch, and gum arabic used in many other trace analyses can be similarly purified by ion exchange. In order to eliminate the interference of calcium and iron rapidly in the colorimetric analysis of silica traces, Lagerstrom, Samuelson, and Scholander (*15*) employed a sulfonic acid exchanger in the hydrogen form.

DETERMINATION OF CONCENTRATION

The determination of total electrolyte concentrations by ion exchange has become of considerable interest. Because it is possible to convert a neutral solution of an electrolyte completely into the free acid on passage through a column of a sulfonic acid exchanger in the hydrogen form, several investigators have studied this method for various electrolyte mixtures. Blumer (*1*) has employed this method for ascertaining the total concentration of natural water supplies and Tolliday, Thompson, and Forman (*25*) have adapted this procedure to chrome tan liquors.

DETERMINATION OF EQUILIBRIUM CONSTANTS AND ACTIVITY COEFFICIENTS

Continuing the work of Vanselow (*30*) and Schubert (*22*) on the use of ion exchange in equilibrium constant and activity coefficient measurements, Mayer and Schwartz (*17*) and Connick and Mayer (*7*) have determined the relative activity coefficients of cerous salts and the association between cerous ions and the perchlorate, nitrate, sulfate, iodide, bromide, fluoride, sulfite, phosphate, and pyrophosphate anions. The technique involves the measurement of the comparative ion exchange equilibrium of the cerous ion and the cation exchange resin in the presence of the foregoing anions.

ELECTRON EXCHANGE RESINS

Although no analytical methods have made use of the "electron exchange" resins of Cassidy (*2*), the successful synthesis of polyvinylhydroquinone by Cassidy and his students (*29*) may lead to a new analytical tool similar to the Jones zinc reductor or the silver modification of this reductor. Cassidy and his students have found resins of this type to form a reversible oxidation-reduction system for the ceric-cerous and bromine-bromide equilibria. Although far from being perfected, these resins may be of considerable analytical significance in the future.

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INDICATORS

I. M. KOLTHOFF, *University of Minnesota, Minneapolis, Minn.*

SINCE the publication of the pioneer work of Sörensen (1909) and Clark and Lubs (1917) no striking new developments have been made in the field of acid-base indicators. As long as we confine ourselves to aqueous media, the present choice of acid-base indicators is more than adequate to satisfy all needs. The situation is different for nonaqueous solutions. No fundamental contributions to adsorption indicators have been made since Fajans (1924) and those who followed his lead published in a series of papers the theoretical and practical fundamentals of the subject. The situation is somewhat different in the field of oxidation-reduction indicators, where there is still a need for more reversible indicators in the relatively high oxidation potential region. More particularly, there is still a dire need for indicators which give a sharp and reversible color change in titrations with bromine (bromate and bromide in acid medium).

There is no justification for presenting every year an annual review in the field of indicators unless there are some fundamental new contributions. A paper by Schulek and Somogyi (*5*) dealing with the constitution and indicator properties of some azo dyes is such an important contribution, worthy of the attention of all analytical chemists. In 1939, Schulek and Rozsa (*4*) described the properties of 4′-ethoxy-2,4-diaminoazobenzene (*p*-ethoxychrysoidin) as an acid-base, adsorption, and oxidation-reduction indicator. To the reviewer's knowledge no application of this indicator has been made yet in this country, although its great usefulness, especially as oxidation-reduction indicator, has been established. The red color of the indicator in acid medium turns faint yellow upon addition of a trace of ceric sulfate or permanganate, the oxidation potential being equal to +0.76 volt and the color change being reversible. More interesting from a practical viewpoint is the behavior toward bromine. In a titration with bromine the red color of the indicator becomes more and more intense as a result of bromination, and at the end point the red color disappears suddenly as a result of a reversible oxidation. In this respect the indicator is markedly different from other azo indicators, like methyl orange and methyl red, which have been used for many years for the detection of bromine but which give an irreversible color change.

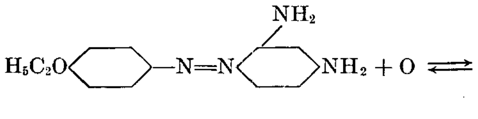
Interesting also is the behavior of *p*-ethoxychrysoidin as an adsorption (and desorption) indicator. In neutral or weakly alkaline medium the indicator has a yellow color, which is not affected by iodide or thiocyanate. Upon addition of silver nitrate the mixture turns wine-red, the intensity increasing with progress of the titration. A sharp color change to yellow occurs at the end point. The indicator is a weak base whose cations are red. Apparently, the silver iodide or thiocyanate, which is negatively charged by iodide or thiocyanate ions, adsorbs the indicator in the cationic form (Gegen ion of the iodide or thiocyanate). At or immediately after the end point the precipitate acquires a positive charge by adsorption of silver ions and the indicator is desorbed, yielding a yellow color in the solution. The color change is perfectly reversible, and it is a simple matter to titrate silver with iodide or thiocyanate, using *p*-ethoxychrysoidin as an adsorption indicator (color from yellow to red).

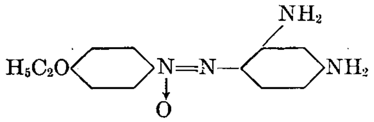
Schulek and Somogyi (*5*) have prepared a number of substituted azo dyes in which especially the effect of OH, OCH_3, OC_2H_5, OC_6H_5, CH_3COO, NH_2, $N(CH_3)_2$, COOH, CH_3, and Br as substituents was investigated. The results are summarized in Table I. All the substances are weak bases. The structural changes occurring in the transformation of the alkaline to the acid form are similar to those of classical azo indicators, like dimethylaminoazobenzene, and need no further discussion here. The color of the acid form reported in Table I is that in 0.01 *M* hydrochloric acid. Some of the compounds, like Nos. 14 and 15, are such weak bases that they do not exhibit an acid color at pH 2. They show a red or orange color in stronger mineral acid solutions.

Virtually all the compounds listed are insoluble in water and are used in the form of a 0.2% solution in ethanol. Considering the slight solubility in water, the reviewer suspects that even those compounds which exhibit a pronounced color change within a relatively narrow pH range are not particularly suited for colorimetric pH measurement. From the data given in the table it is readily seen which indicators are suitable for certain acid-base titrations. None of the compounds seems to have any distinct advantages over existing indicators in acid-base titrations.

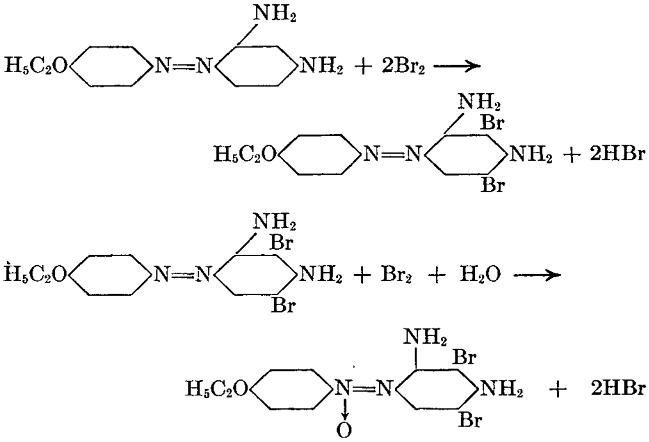
The significance of the publications of Schulek and Somogyi is derived mainly from the contribution to reversible oxidation-reduction indicators and especially reversible indicators in titrations with bromine in acid medium. Compounds 3, 4, 5, 6, 7, 11, 19, 26, and 27 are suitable for titrations with ceric sulfate and bromine in acid medium, but 28 and 30 are suitable only in ceriometry. Several indicators which do not contain bromine are brominated during a titration, which results in an intensification of the red color.

Both with ceric sulfate (or permanganate) and bromine the reversible color change from red to yellow is due to an oxidation of the azo to an azoxy compound:





In titrations with bromine, bromination of the reduced (azo) form of the indicator occurs before the end point.



The oxidation potentials of the compounds which are suitable as oxidation-reduction indicators are not listed. It would be of interest to know in what respect various substituents affect the oxidation potential.

Of practical importance is the proper acidity in titration with ceric cerium and with bromine. If the acidity is too great many of the azo compounds can be oxidized in an irreversible way. On the other hand, in many oxidation reactions a high acidity is desirable. For this reason compounds 20, 21, 22, 23, 24, and 25 are unsuitable in bromatometry. Results are given of the titrations of ferrous sulfate solution in about 1 *N* sulfuric acid with 0.005 *N* ceric sulfate using 1 drop of 0.2% indicator solution, in a final volume of about 40 ml. The concentration of iron varied between 0.0012 and 0.012 *N*. Using indicators 3, 4, 5, 6, 7, and 11 excellent results were obtained; the indicator correction was of the order of −0.05 ml. of 0.005 *N* ceric cerium.

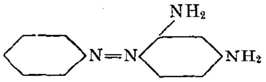
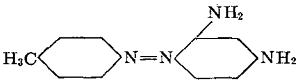
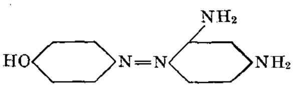
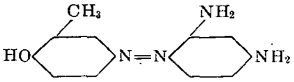
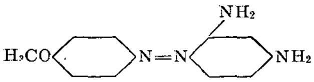
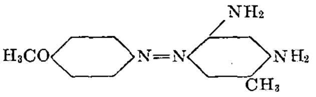
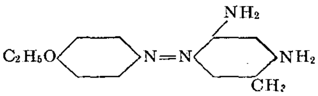
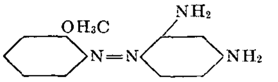
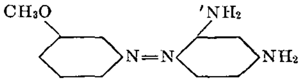
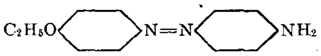
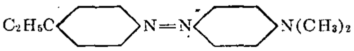
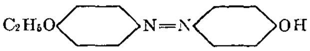
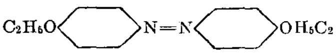
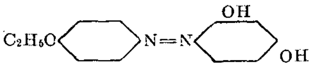
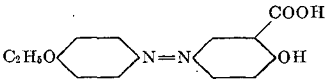
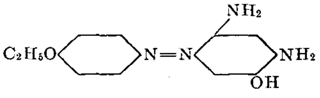
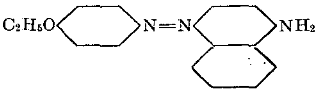
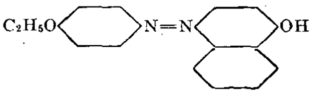
The indicator correction was also determined in titrations of L-ascorbic acid in about 1 *N* hydrochloric acid with bromate; the solution was 0.5% in potassium bromide and final volume about 40 ml. With indicators 3, 4, 5, 6, 7, and 11 the indicator correction corresponded to about −0.07 ml. of 0.005 *N* standard reagent solution. No statement is made of the stability of the oxidized form in the presence of an excess of oxidizing agent.

Compounds 2, 3, 4, 5, 6, 7, 8, 17, 19, 22, 26, 28, 29, and 30 are useful as adsorption and desorption indicators. Compounds 3, 4, 5, 6, 7, and 19 have a multiple function as indicators; they are suitable as acid-base, oxidation-reduction [Ce(IV) and Br_2], and adsorption (desorption) indicators.

Belcher (*1*) recently compared in titrations with bromate *p*-ethoxychrysoidine as indicator with α-naphthoflavone which had been recommended by Uzel (*8*) and also by Schulek (*3*) and with fuchsin (see Raykhinstein and Kocherigina, *2*). According to Belcher the naphthoflavone gives the sharpest end point from a very pale straw color to a deep brownish orange. The stock solution contains 0.1% flavone in ethanol, 0.3 to 0.5 ml. of indicator being used per 50 ml. of solution titrated. The color change is reversible; an excess of arsenic trioxide restores the original color. A direct titration of 8-hydroxyquinoline solutions with bromate using naphthoflavone as indicator is too slow to be practicable. Apparently, Schulek prefers *p*-ethoxychrysoidine over naphthoflavone, which agrees with the reviewer's choice.

A valuable expansion of the ferrous phenanthroline series of indicators has been made by Smith and Brandt (*6*) by the addition

Table I. Properties of Azo Dyes as Indicators

No.	Name	Constitution	Color		Color Change Interval, pH	Oxidation-Reduction Property, Color		
			Acid, pH 2	Alkaline		Oxidized	Reduced after Oxidation with	
							Ce(IV)	BrO_3^-
1	Chrysoidin		Orange-yellow	Yellow	4.2–6.0	Weak yellow	....	...
2	4'-Methyl-2,4-diaminoazobenzene		Red	Yellow	4.2–6.2	Weak yellow	....	...
3	4'-Oxy-2,4-diaminoazobenzene		Red	Yellow	3.4–5.4	Weak yellow	Red	Red
4	4'-Oxy-3'-methyl-2,4-diaminoazobenzene		Red	Yellow	3.8–5.8	Weak yellow	Red	Red
5	4'-Methoxy-2,4-diaminoazobenzene		Red	Yellow	4.4–6.4	Weak yellow	Red	Red
6	4'-Methoxy-2,4-diamino-5-methylazobenzene		Red	Yellow	4.9–6.7	Weak yellow	Red	Red
7	4'-Ethoxy-2,4-diamino-5-methylazobenzene		Red	Yellow	4.8–6.6	Weak yellow	Red	Red
8	2'-Methoxy-2,4-diaminoazobenzene		Orange-yellow	Yellow	4.2–6.0	Weak yellow	Weak orange	...
9	3'-Methoxy-2,4-diaminoazobenzene		Orange-yellow	Yellow	4.0–5.8	Weak yellow	....	...
10	4'-Ethoxy-4-aminoazobenzene		....	....	.....	Weak yellow	Violet, in 50% H_2SO_4 blue	...
11	4'-Ethoxy-4-dimethylaminoazobenzene		....	....	.....	Yellow	Violet	Violet
12	4'-Ethoxy-4-oxyazobenzene		Light yellow	Yellow	6.0–8.0	Green-yellow	Red	...
13	4'-Ethoxy-4-ethoxyazobenzene		Light yellow	Yellow	.....	Light yellow	Red	...
14	4'-Ethoxy-2,4-dioxyazobenzene		Light yellow	Yellow	7.0–9.0	Light yellow	Red	...
15	4'-Ethoxy-3-carbonic acid-4-oxyazobenzene		Light yellow	Yellow	.....	Light yellow	Orange	...
16	4'-Ethoxy-5-oxy-2,4-diaminoazobenzene		....	....	.....	Light yellow	Red	Red
17	Phenetole (4-azo-4)-1-naphthylamine		Violet	Yellow	2.2–4.0	Light yellow	Red	...
18	Phenetole (4-azo-4)-1-naphthol		....	....	7.2–9.0	Light yellow	Red	...

(*Continued on next page*)

Table I. *Continued*

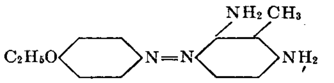
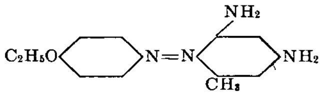
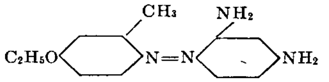
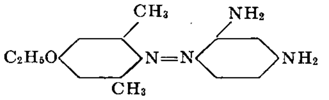
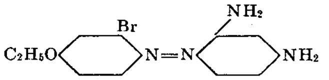
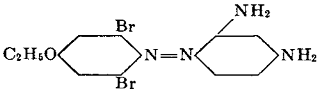
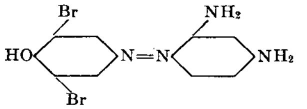
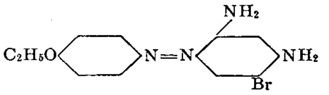
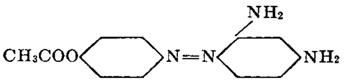
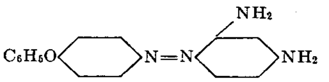
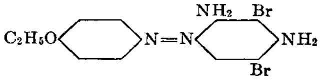
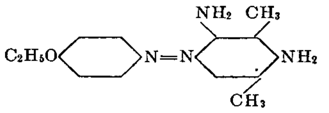
No.	Name	Constitution	Color		Color Change Interval, pH	Oxidation-Reduction Property, Color		
			Acid, pH 2	Alkaline		Oxidized	Reduced after Oxidation with	
							Ce(IV)	BrO_3^-
19	4′-Ethoxy-2,4-diamino-3-methylazobenzene		Yellow	Red	.....	Light yellow	Red	Red
20	4′-Ethoxy-2,4-diamino-6-methylazobenzene		....	....	.....	....	....	...
21	4′-Ethoxy-2′-methyl-2,4-diaminoazobenzene		....	....	.....	....	....	...
22	4′-Ethoxy-2′,6′-dimethyl-2,4-diamino-azobenzene		....	....	.....	....	....	..
23	4′-Ethoxy-2′-bromo-2,4-diaminoazobenzene		....	....	.....	....	....	...
24	4′-Ethoxy-2′,6′-dibromo-2,4-diaminoazobenzene		....	....	.....	....	....	...
25	4′-Oxy-3′,5′-dibromo-2,4-diaminoazobenzene		....	....	.....	....	....	...
26	4′-Ethoxy-2,4-diamino-5-bromoazobenzene		....	....	.....	Weak yellow	Red	Red
27	4′-Acetoxy-2,4-diaminoazobenzene		....	....	.....	Weak yellow	Red	Red
28	4′-Phenoxy-2,4-diaminoazobenzene		....	....	.....	Weak yellow	Red	...
29	4′-Ethoxy-2,4-diamino-3,5-dibromoazobenzene		....	....	.....	Weak yellow	Red	...
30	4′-Ethoxy-2,4-diamino-3,5-dimethylazobenzene		....	....	.....	Weak yellow	Red	...

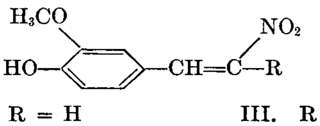
Table II. Properties of Indicators

Name	Designation	Melting Point, ° C.	Color Change Interval, pH	pK_I	Rate of Fading in 0.1 *N* NaOH, $t_{1/2}$ Min.
4-Hydroxy-3-methoxy-β-nitrostyrene	I	169	6.1–8.1	7.6	1.3
1-(4-Hydroxy-3-methoxyphenyl)-2-nitro-1-propene	II	103	6.8–8.8	8.2	7.2
1-(4-Hydroxy-3-methoxyphenyl)-2-nitro-1-butene	III	80.5	6.8–8.7	8.1	11.8
1-(4-Hydroxy-3-methoxy)-α′-nitrostilbene	IV	125	6.4–8.4	7.7	5.2

of ferrous tri-(5,6-dimethyl-1,10-phenanthroline) called dimethyl ferroin. A 0.01 *M* solution is prepared by reaction of 0.03 mole of the base with 0.01 mole of ferrous solution. In 1 *N* acid the oxidation potential is 0.97 volt. The indicator gives a sharp end point in the titration of ferrous iron with dichromate in 1 to 2 *F* (formal) hydrochloric or sulfuric acid. The color change is from red to greenish and completely reversible.

ACID-BASE INDICATORS

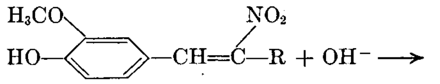
The compound 4-hydroxy-3-methoxy-β-nitrostyrene (I) was recommended in 1925 by M. G. S. Rao *et al.*, as acid-base indicator, but no quantitative data on its properties were given. Stewart and Clark (7) prepared and investigated compound I and some of its homologs by condensing vanillin with the appropriate nitroparaffin.



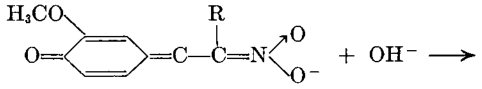
I. R = H
II. R = CH_3
III. R = C_2H_5
IV. R = C_6H_5

In extremely dilute solutions the color change is from faint yellow (acid) to pink (alkaline) for I and IV and from faint yellow to amber for II and III. In strongly alkaline medium the color fades, but the sluggishness of this color change renders it useless for indicator purposes. The properties of the compounds are summarized in Table II.

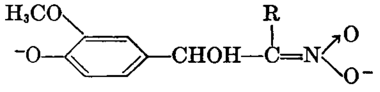
The mechanism of the color change is given by:



Faint yellow



Pink or amber



Colorless

Stock solutions (0.1%) in alcohol are prepared. It is not stated whether any of the indicators have advantages over phenol red or other Clark and Lubs indicators which have about the same color change interval.

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FLUOROMETRIC ANALYSIS

CHARLES E. WHITE, *University of Maryland, College Park, Md.*

ALTHOUGH this review is concerned primarily with the application of fluorescence to analytical problems, it is worth while to call attention to a book on fluorescence and phosphorescence by Pringsheim (*57*). This is a much enlarged and revised edition of the author's previous book on luminescence. The analyst will find of particular interest the discussion on the theory of fluorescence, and the sections on the fluorescence of organic compounds and the luminescence of pure inorganic compounds.

The Russian Academy of Science has published a book on luminescence analysis, edited by Konstantinova-Shlezinger (*39*). Because this is entirely in Russian, it will find but little use in America until a translation appears. This same author has given a short review on fluorescence analysis (*38*). Feigl (*22*) devotes Chapter XII of his book on the "Chemistry of Specific, Selective and Sensitive Reactions" to the analytical use of fluorescence effects. This is so brief that many applications are merely named and much recent work is not included.

In a new book on "Methods of Quantitative Analysis," Milton and Waters (*48*) give a short discussion of fluorometric analysis with directions for the analysis of some few elements. Unfortunately, their references on this subject, except in one case, do not go beyond 1943. A critical review of fluorescence microscopy has been given by Hamperl (*29*).

APPARATUS

In a similar review last year (*71*), the 360 B.L. phosphor lamps were mentioned as a new source of ultraviolet radiation for fluorometric work. These have now been improved to give over twice the ultraviolet output of the earlier models and the maximum is now at about 3500 A., whereas the former was at 3600 A. Curves giving a comparison of these lamps have been published in a bulletin of the Sylvania corporation (*69*). Analysts using the Klett fluorometer will be interested in an article by Slater and Morell (*67*) in which are given many suggestions on the use of this instrument, especially with reference to its use in determining thiamine and riboflavin. The use of the Pfaltz and Bauer instrument has been smplified by a test tube adapter which has been described by Durst and Lewis (*21*) and Myers (*50*).

In spite of the efforts of commercial manufacturers, many analysts prefer to build their own fluorometers. Price (*56*) and others, in a report from the Atomic Energy Commission, describe an instrument which they have designed to determine uranium by the fluorescence of the uranium-sodium fluoride melt. Their present instrument is of simple design, using a photomultiplier tube. It is sensitive to 10^{-11} gram of uranium and the authors indicate that a still more desirable instrument will be described in a forthcoming publication.

A simply constructed photoelectric colorimeter and fluorometer is described by McGillivray (*45*). For photographing the fluorescence spectrum, Scheminsky (*64*) uses a hand spectroscope and a Contax camera, and reports excellent results. Fluorescence microscopy has many applications and a simple device including types of filters for this purpose is described by Zamkov (*72*).

INORGANIC APPLICATIONS

An interesting technique for the identification of the cations by using paper chromatography in conjunction with fluorescence has been reported by Pollard (*55*) and his co-workers. The cations are dissolved in a solvent consisting of water, butanol, acetic acid, and acetoacetic acid ester, or other appropriate mixtures. This is dropped on Whatman No. 1 filter paper, so as to give spots about 1.3 cm. in diameter and 4 cm. apart. The paper is then sprayed with morin, oxine, etc., and observed under ultraviolet light. Twenty-four cations can be immediately detected by this procedure.

The quantitative determination of oxygen (*40*) in quantities of 0.01 to 20 p.p.m. can be accomplished by permitting it to oxidize leucofluorescein to fluorescein. Stable solutions of the reagent are prepared by reducing fluorescein with sodium amalgam and storing it under a light petroleum fraction. As little as 1×10^{-11} mole of oxygen (*33*) can be detected by its effect on trypaflavin adsorbed on silica gel. Oxygen destroys the orange fluorescence of this compound and a greenish hue appears.

Several references to the analysis of beryllium in rocks were reported in this review last year. Sandell (*63*) has made some revision of his method in which morin is used as the reagent and shows that as small an amount as 0.05 microgram of beryllium can be identified. Granite containing as low as 3 p.p.m., was analyzed successfully.

Rodden (*60*) has described a fluorometric method for uranium which detects as little as 10^{-11} gram using the sodium fluoride flux technique. This same technique has been investigated by Price (*56*) and his co-workers, who have made an extensive study on the effect of inhibitors on the uranium fluorescence. Gibbs and Evans (*25*) describe a portable instrument for measuring the fluorescence in this procedure. Boutaric and Maraux (*9*) have studied the effect of inhibitors on uranium solutions. This is of obvious interest to analysts using the solution method.

The rare earths (*49*) may be detected in coal ash in quantities which are too small for spectrographic analysis by mixing the ash with calcium oxide and subjecting it to cathode rays. The resulting luminescence is so bright that an exposure of only 5 to 12 minutes is necessary to photograph its spectra.

In the mineralogical field Cannon and Murata (*13*) have shown that the molybdenum content of scheelite can be rapidly determined by comparing the fluorescence of the sample with a standard. The fluorescence of mineral zircon is claimed by Foster (*23*) to have considerable practical significance in petrological problems. A new technique for observing the fluorescence of minerals with a microscope or spectroscope is described by Komovskiĭ (*37*).

The luminescence of optical glass (*11*) under ultraviolet excitation provides a means of rapidly analyzing specimens for classification into commercial types.

Sill and Peterson (*66*) have described a unique and useful qualitative method for thallium, which employs the bright blue fluorescence of the thallous ion, in sodium chloride solution, under the influence of short-wave ultraviolet light. The sensitivity is 1 in 50,000,000 and there are no ions that give a similar fluorescence under these conditions.

APPLICATIONS IN BIOLOGICAL CHEMISTRY

A general review of the biological applications of fluorescence analysis has been given by Radley (*58*).

The fluorometric determination of adrenaline in the blood has been the subject of considerable study. Block (*7*) points out that the blood contains an inhibitor for the fluorescence of adrenaline which can be removed by dialysis and is destroyed by prolonged boiling or by ultraviolet irradiation. Annersten (*3*) and his co-workers show that determinations made, using the dialysis method, give results closely corresponding to other methods. Pekkarinen (*53*) uses aluminum hydroxide to adsorb adrenaline before applying the reaction to produce the fluorescence. The fluorometric methods for adrenaline are discussed by Lund (*44*).

The quantitative fluorometric determination of estrone and estradiol has been developed by Jailer (*32*), who shows a linear relationship between the fluorophotometer readings and the concentration of these compounds in sulfuric acid solutions. Boscott (*8*), by using suitable solvents with the phosphoric acid test for natural estrogens, has obtained characteristic color or fluorescent reactions for several of these and has developed a quantitative procedure for dienestrol. Mariani (*46*) uses the Pulfrich fluorophotometer for determining estrone in the range of 6 to 30 micrograms.

The determination of folic acid by the fluorescence of its oxidation product has become an established procedure. Allfrey (*1*) and others show that interferences may be removed by adsorbing the oxidation product on Florisil and eluting with borax solution. Application of the procedure shows good agreement with the microbiological method. Andreeva and Bukin (*2*) indicate that for high protein materials an enzyme treatment is necessary to liberate all of the folic acid.

The estimation of nicotinamide in the presence of other nicotinic acid derivatives is accomplished by Chaudhuri and Kodicek (*15*) by treating the sample with cyanogen and permitting it to stand in an alkaline medium to form the fluorescent compound. Results are given for the analysis of various organs, wheat products, and yeasts. A method for the determination of the 6-pyridone of *N*′-methylnicotinamide in urine has been reported by Rosen (*61*) and others.

The fluorescent spectra of 3,4-benzopyrene has been studied by Bergol'ts (*5*) and his associates as a means of identification in the animal organism. This method has been used for detecting benzopyrene in the urine of mice after 0.25 mg. had been injected subcutaneously.

The analysis of pamaquine in blood plasma is carried out in concentrated sulfuric acid solution. This serves for a range of 10 to 500 micrograms of S.N.-13276 and S.N.-3294 and is applicable even when these substances are mixed with quinine (*31*).

Fluorometric techniques in the identification and determination of porphyrins are in general use. Kliewe (*34*) shows that the intensity of the fluorescence of these compounds is greater in phosphoric acid than in hydrochloric. Comfort (*18*) has examined over 3000 molluscan shells, some of which were over 100 years old, and has shown that the red fluorescence of porphyrin is just as strong in these as in fresh material.

Riboflavin decomposition products have been studied by paper partition chromatography and subsequently examined under ultraviolet light by Hais and Pecáková (*28*). The characteristic fluorescence of lumiflavin and lumichrome was observed in addition to other new spots. De Ritter (*20*) and others give a critical review of the methods for the determination of riboflavin in urine. This determination is also described by Wang (*70*) and his associates. The determination of riboflavin in blood has been dealt with by several authors (*12, 26, 27*); in some cases it is recommended that the total fluorescence and also that remaining after the addition of hydrochloric acid be determined. The riboflavin fluorescence is quenched by the acid and is determined by difference. Bessey (*6*) and his associates have devised a method for determining the nucleotides of riboflavin in biological material and give comparison data for the fluorometric and enzymatic methods. The former is much simpler and equally as accurate as the latter. By use of a sensitive fluorometer Kodicek and Wang (*36*) have determined the riboflavin content of various meats and vegetables without concentration by adsorption.

Salicylates in the blood may be determined by the strong blue fluorescence in alkaline solution. Proteins are removed by precipitation with tungstic acid and Saltzman (*62*) has obtained excellent recovery of salicylate over a range of 0.02 to 0.5 mg.

The fluorometric determination of thiamine by the tentative A.O.A.C. method has been the subject of a collaborative study and found to give good results. It is recommended that the method be made official (*35*). Patrick and Wright (*52*) recommend the use of mercuric oxide as an oxidant in the analysis of pharmaceutical products for thiamine. Their method is apparently more rapid and gives more accurate results than where ferricyanide is used. Ridyard (*59*) gives a number of refinements that experience has shown to be of utmost importance for the attainment of speed and accuracy in the determination of thiamine in wheat products. Mawson and Thompson (*47*) show how to avoid the interference of nicotinamide methyl chloride in urine analysis and Bowman (*10*) has outlined a method for determining thiamine in potatoes.

Newman (*51*) and his associates have applied the sodium fluoride fusion method to the determination of uranium in biological materials. As little as 0.005 microgram of uranium per gram of tissue may be detected.

The use of fluorescent dyes as tracers in biology and medicine has been reviewed by Lange (*41*). This article is written in a popular style and covers the use of fluorescence in ophthalmology, blood circulation, etc.

Analysts interested in the determination of chlorophyll will find much pertinent information in an article by Livingston (*43*). He shows that chlorophyll dissolved in pure hydrocarbons is practically nonfluorescent. The addition of 0.01% more water, alcohol, or amines raises the intensity of the fluorescence to the same as when alcohol is used as a solvent. The absorption spectrum of chlorophyll in these solvents is also given.

FLUORESCENCE IN ORGANIC ANALYSIS

A quantitative determination of citric acid, based upon its transformation into the highly fluorescent compound ammonium citrazinate, has been developed by Leininger and Katz (*42*). From 10 to 75 micrograms of citric acid give good results even in the presence of tartaric and malic acids. The application to the analysis of citrus fruit juices is described.

Glycerol in dilute aqueous solutions can be easily identified by a reaction with 2,7-naphthalenediol in concentrated sulfuric acid. The aldehyde acrolein, formed from the glycerol, condenses with the reagent and forms a yellow to reddish yellow solution which shows a strong green fluorescence (*24*).

Purine and pyrimidine derivatives can be identified on chromatogram spot test paper (*30*) if excited with wave motions in the order of 2500 A.

The reaction of malic acid and succinic acid with resorcinol in concentrated sulfuric acid has been used by Barr (*4*) to determine these acids. When the solution is made alkaline it becomes highly fluorescent.

Phillips (*54*) has described a method of identifying α-amino acids and dipeptides by examination of chromatograms under ultraviolet light.

The fluorescent spectra of many polycyclic aromatic hydrocarbons in solution have been determined by Schoental and Scott (*65*). A correlation is shown between the position of the spectrum and the number of quinoid rings in the structure.

ANALYSIS OF PHARMACEUTICAL PRODUCTS

Chase and Pratt (*14*) have developed a system of identification for powdered vegetable drugs by means of their fluorescence directly or after treatment with sodium hydroxide. Alcohol extracts were also used. One hundred and fifty-one vegetable drugs were studied and useful data are tabulated.

The fluorometric determination of the ketotetrahydropyridenes in urine has been improved by De Ritter (*19*) and his associates, so that good results are obtained at excretion levels as low as 1 mg. per day.

Antrycide, a new trypanciodal drug, may be determined in blood plasma by its reaction with eosin, wherein the eosin salt of the alkaloid is extracted with butanol and chloroform and measured in a fluorometer (*68*).

A fluorescence study of Bonitreager reaction for anthraquinone drugs has been made by Christensen and Abdel-Latif (*17*). The authors conclude that the color of the fluorescence is specific for each of the drugs containing emodin and can be used in their identification. In order to increase the ease of observation, these same authors advocate the use of capillary tubes as containers for the solutions (*16*).

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INSTRUMENTATION

RALPH H. MÜLLER, *New York University, New York, N. Y.*

THE advances in instrumentation continue to be derived from every branch of science and technology, and they all hold promise for the solution of the analyst's problems. It is particularly profitable to seek suggestions for new analytical techniques in wholly unrelated fields, and this is possible especially if one examines new developments in the light of their instrumental possibilities. Thus a new trick in rocket guiding, telemetering, or automatic navigation may have direct bearing on an analytical problem. More frequently than not, the fantastic conditions under which some of these devices are required to perform reliably lead to highly original and ingenious solutions and it becomes possible to apply them with greater ease to the comparatively ideal conditions of the laboratory.

If we examine current developments and try to discover a characteristic trend, it seems to lie in a distinct desire to produce instruments that are inherently foolproof and self-contained. The modern user of instruments is very properly becoming increasingly intolerant of accessories such as thermostats, constant-voltage transformers, calibration curves, and other symptoms of incomplete design. There seems to be no end to the possible improvements of existing instruments in this respect. Although few analysts will agree that all analytical instruments should record the results automatically and in addition collate the data, many instrument designers are thinking precisely in these terms. It is possible that we need a clearer distinction between scientific apparatus and instruments. The distinction is subtle and probably incapable of definition at the present time, but seems to imply that apparatus requires skilled manipulation, whereas an instrument may largely assume human functions including elementary judgment and discretion.

At a time when many analysts are beginning to view the vast array of instruments with increasing dismay and to wonder what is coming next, it is encouraging to note that we are rapidly approaching a "steady state" wherein an assembly, however complicated, may be resolved into standard components. All but one of these, the primary conversion element, we may expect to see in the near future as standard packaged items. This has been pointed out very clearly by Condon (*7*) in a recent article on the role of instrumentation. It may be useful to develop this argument with the aid of Figure 1.

The phenomenon or quantity x, which is to be evaluated and employed in some fashion by the instrument, requires an appropriate conversion element and preferably one that will develop an electrical signal in response to x. The output of the conversion element is amplified to a high degree and then fed to a power unit or servosystem. A portion of the output, which may be electrical, a shaft rotation, or other signal, is returned to the input in a degenerative or compensating sense. Under these conditions, it can be shown that the accuracy of the system depends very little upon amplifier gain, frictional losses, etc. For the sake of completeness, the diagram is supplemented by symbols representing a computer, recorder, and controller element. In actual practice, these may well be incorporated in other parts of the system and certainly, in the case of the controller, the feedback loop would originate in the object or device under control. The computer element, whether simple or complex, may be used to generate any desired function of x, or it may combine x or $f(x)$ with information from one or more other servosystems. The function of the recorder is obvious, although in special cases it may derive its commands from the computer.

Packaged amplifiers and complete servosystems are already available and the high-speed electronic recorder is a commonplace in the research laboratory. The same is true of controllers, and indeed many of these are in themselves self-contained servosystems. Computer elements are also available, but obviously they do not lend themselves readily to standardization. It can be seen, therefore, that every element in Figure 1 to the right of the conversion element is susceptible to some degree of standardization and incorporation into one packaged unit.

The principal and continuing problem of the analytical instrument designer will be to develop new detectors and conversion elements to solve his problems. The rest is a matter of engineering. Several of the examples chosen to illustrate instrumentation progress have been selected to emphasize these trends.

PRIMARY ELEMENTS

The search for new or improved primary conversion elements continues unabated and each improvement at once brings a better performance to existing instruments or inaugurates a new technique. There have been few essentially new elements, but considerable improvement in those already known. Several ingenious applications have been described which at once suggest several uses of interest to the analyst.

A rather startling development is the electrolytic thermistor. Aside from its practical uses, the phenomenon could well supply the electrochemist with a score of research problems.

According to Gutman and Simmons (*15*), the thermistor consists of two platinum electrodes immersed in a water glass solution of specific gravity 1.38. The electrolyte is contained in a thin-walled U-tube. In the region of 300° K. the system has a half-temperature value of 7.2° C. This mode of designating thermistor characteristics means that a temperature rise of 7.2° C. will halve the resistance. As might be expected, alternating current must be applied to the system to avoid electrode polarization. Relatively enormous potentials can be applied to attain high differential sensitivity; the authors have used 240-volt alternating current. The cold resistance can be controlled by dimensional factors, and the temperature coefficient by dilution. Measurements may be made with a bridge circuit or more directly by passing constant-current alternating current in series with a "swamping" resistor (100 × thermistor resistance) and measuring the potential difference across the thermistor with a vacuum-tube voltmeter.

Electrolytic thermistors show somewhat greater lag than ordinary solid thermistors. Presumably, this is solely a matter of heat transfer and therefore a function of design and dimension. The authors have examined a few other electrolytes, none of which is as effective as water glass. It has been known for a long time that several electrolytes in mixed solvents exhibit large temperature coefficients of resistance. This could easily become a refreshingly interesting field of investigation for physical chemists who have tired of compiling activity coefficients or reaction velocity constants.

The use of semiconducting ceramic glazes for measuring temperature has been described by Forrest (*9*), who shows that a porcelain rod can be coated and fired with a suitable glaze. Silver terminals are affixed and the system exhibits typical thermistor behavior in which the logarithm of the resistance is a linear function of reciprocal temperature.

A portable thermistor bridge for micrometeorology among growing crops has been described by Penman and Long (*31*), which measures temperature and humidity (wet- and dry-bulb style) and may be used as an anemometer. An elegant and extremely well designed arrangement for measuring small temperature changes in liquids, devised by Greenhill and Whitehead (*12*), is used at present for measuring the heats of wetting and adsorption of metal powders in nonpolar solvents and long-chain compounds dissolved therein. Changes of the order of 0.01° C. are measured and recorded to ±0.0002° C.

A thermistor is used in one arm of a direct current bridge. The unbalance of a mirror galvanometer illuminates a phototube with light modulated at 5000 cycles. The signal is amplified 4 × 10^4-fold and the alternating current component is fed back to an indirectly heated thermistor located in an adjacent arm of the bridge. The feed-back component is rectified by a crystal rectifier and registered on a 0 to 5-ma. recorder.

At the present time, no extensive data are available on the reliability and strict reproducibility of thermistors. Furthermore, this question seems to be a source of continuing concern for the manufacturers of those devices. However desirable such stabilization and reproducibility may be, they will not deter the enterprising investigator from utilizing some measure of short-term stability, and throwing the burden of reliability upon some null method or feedback scheme of measurement. Experience gained in the author's laboratory indicates that the extraordinary sensitivity of thermistors makes them eminently suitable for thermometric titrations, cryoscopy, radiometry, and temperature compensation (*27*). The principal challenge seems to lie in the elimination of ambient effects and dependence upon long-term stability. These are largely matters of correct instrument design.

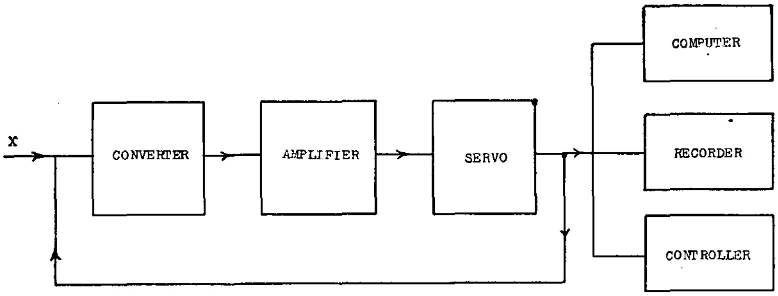

Figure 1. Standard Components of Instrumentation Assembly

Automatic temperature compensation is taken for granted in certain branches of instrumentation, notably in airborne equipment. There is probably no legitimate excuse for tolerating the need of temperature correction in any of the chemist's measurements, and this would seem to be a promising field of investigation for the instrument designer. A comprehensive theoretical treatment is badly needed, particularly theorems on compensating networks employing nonohmic temperature-sensitive elements like thermistors. Their nonlinear behavior affords unusual possibilities, especially in combination with elements of zero or positive temperature coefficient.

An excellent example of this class of compensation is the recent work of Moneypenny (*24*) who describes equipment for the measurement of electrolytic conductivity. His design is also highly original from the electrical standpoint. The usual bridge circuit employed in electrolytic conductance measurements requires simultaneous establishment of resistive and reactive balances, of which only the former is of interest. In this method, use is made of the boundary conditions between stability and instability in an amplifier in which the actual measuring bridge provides a feed-back network. The transition from quiescence to oscillation is governed by the resistive balance condition only. Visual indication of the transition is provided and the instrument can be made direct-reading. Temperature compensation is provided by a thermistor in the conductance cell. The equipment has been applied to the determination of sulfur and carbon in steel, in the former by absorbing sulfur dioxide in a stabilized hydrogen peroxide solution in the conductance cell and in the latter by absorbing carbon dioxide in potassium hydroxide solution. The measurements are good to 0.001% carbon.

Thermistor elements have also been used as radiometric devices. One advantage over thermocouples lies in a greater ease of impedance matching to amplifiers. This is not the sole criterion of suitability; for a more complete comparison the reader is referred to the excellent review on infrared techniques by Williams (*40*). Fowler (*10*) describes a rapid infrared gas analyzer which is particularly suited for physiological carbon dioxide determinations, for following the progress of anesthesia and related studies. The instrument employs a Nichrome source run at red heat, crossed beams, and selected presensitizing filters with twin thermistors as detectors.

A second cousin to the thermistor is another member of the semiconductors class of devices, the transistor. The full resources of solid state physics are being applied to a study of this device, which consists essentially of a polished plate of germanium, upon which two closely adjacent needle or cat-whisker contacts are made. Input and output circuits consist of each of the contacts with reference to the base plate. With the appropriate potentials and polarities applied to these electrodes, the system possesses amplifying properties.

The present state of development is discussed in an editorial note (*3*) and the physical principles involved in transistor action are described by Bardeen and Brattain (*1*). Three other papers (*16*, *38*, *39*) deal with the mechanism of transistor behavior, and still another by Ryder and Kirchner (*36*) analyzes the circuit aspects of the transistor. In this paper, the device is intentionally discussed as a four-terminal "black box." This standard and useful procedure of the electrical engineer permits one to describe the circuit behavior of any device without reference to its structure or the physical principles that are involved. Included is a comparison between transistors and conventional vacuum tubes. Their behavior is entirely different, but they may be compared in terms of similar tasks which they may be required to perform, such as amplification. In such comparisons, transistors indicate great promise in smaller size, and lower stand-by losses and cost. At present, they do not compare too favorably with vacuum tubes for gain, versatility, and power-handling ability, but as these authors point out, the vacuum tube is about 40 years old and the transistor is a comparative newcomer. As they put it, "Let's not be too hard on the baby."

A definite example of their superiority in certain cases is described by Reich and Ungvary (*34*) in the case of a transistor trigger circuit. Here the abrupt switching action, which is so useful in electronic circuitry, has the advantage over equivalent tube circuits of shorter triggering time, simplicity, small size, absence of filament supply, and low operating voltage. The rise and fall times are of the order of 0.1 microsecond. The trigger is stable from zero triggering frequency up to the maximum. This is about 1 megacycle, but probably will work up to 10 Mc. An output signal of 5 or 6 volts is obtained and the device can be converted to a relaxation oscillator or pulse generator.

Even if transistors were ultimately limited to low power and moderate gain, they would nevertheless be attractive. The outstanding example is in the field of digital computers. Some of these contain as many as 18,000 tubes and the power supply and ventilation problems are a major consideration. Computer experts are therefore greatly interested in developments which promise to remove such limitations. In this connection, the extensive use of printed circuits represents a parallel economy.

PHOTOMULTIPLIERS

There is continued progress in the improvement of photomultiplier tubes, which are finding more uses in photometric problems of all classes. Improved signal-to-noise ratio and a greater reduction in dark current of thermal origin are two of the principal goals. The improvement of scintillation counters employing these tubes is one of the major activities in nuclear measuring techniques. A review on photomultipliers by Rodda (*35*) lists

23 references and deals with some of the latest developments. A head-on type has been developed with a semitransparent photocathode 1.5 inches in diameter and effective area of 1.8 square inches. The response curve is peaked at 4800 A. with cutoffs at about 3100 and 6500 A. There are ten dynodes operated nominally at 90 volts per stage. The normal amplification is about 400,000-fold. It is designated as the R.C.A. 5819.

The spark counter has not found wide use as a radiometric device, but as originally described by Greinacher (*14*) it is extraordinarily sensitive. In his improved arrangement the gap of a few millimeters is between a sharp needle and a polished metal surface acting as the cathode. The device is operated in air with several thousand volts across the gap and a very high resistance in series with it. The photoelectric threshold of the metal constituting the cathode plate determines its spectral response and upon illumination minute sparks jump the gap. The pulses need little amplification for subsequent counting or recording. The counter is also sensitive to ionizing particles and cosmic rays.

The Rosenblum counter is an improvement on this principle, and recent developments in its use as a detector of fast ionizing particles are described by Payne (*30*). Presumably, it is also sensitive to light of the proper wave lengths. Instead of needle to plate, the Rosenblum counter uses a wire to plate arrangement. The advantage over the Greinacher counter is the existence of a definite plateau which should make the counter less susceptible to supply voltage variations. The counter has a dead time of 10^{-4} second and an accidental count of less than 1 in 4 hours. It can detect protons or fast electrons. The counter operates on 5000 volts with a 10-megohm resistor in series. A 400-volt pulse is generated when a spark passes. The separation in time of pulses is 50 to 100 microseconds and the pulse rise time is estimated to be less than 10^{-7} second.

STRAIN GAGES

In last years' review (*25*) some of the uses and advantages of strain gages were described. Progress in their wider use by chemists should be aided by the appearance of an excellent little monograph by Dobie and Isaac (*8*), which gives a detailed account of the best in British and American practice. The book includes very useful tables of commercial gage characteristics as well as related techniques for the measurement and interpretation of stress-strain phenomena.

A simple electrical strain gage for recording small tensions, described by the noted physiologist, A. V. Hill (*18*), consists of a very short length of very fine nickel-chromium alloy wire stretched between an upper pin and a phosphor-bronze spring at the lower end, to which there is also attached a chain, for load application. The total resistance is about 35 ohms and the response is about 0.37% per 100 grams. It will accommodate loads up to 100 grams. The temperature coefficient is 7×10^{-5} per ° C. The response is fast enough for oscillographic recording and the author notes an amusing property of this gage in which the application of a sharp pulse of current to the wire will cause it to sag before the spring can take up the increased length.

The strain gage has few competitors on the basis of simplicity, low cost, and ease of installation. However, on the basis of sensitivity and for the detection of much smaller displacements, a transformer device has now appeared in commercial form with highly reproducible characteristics, which offers distinct advantages.

As described by Macgeorge (*20*) it consists of a compact assembly comprising a primary winding and two secondaries which can be connected in series opposition. If the primary is excited with alternating current there will be effective cancellation in the opposed secondaries and essentially zero signal in the output. A small iron core is arranged to move freely within the coils and, depending upon its position, it can vary the coupling between the primary and the two secondaries. Any resultant asymmetry in the core position will give rise to a preferential transformer action and the output signal will be proportional to core position as well as phase sensitive.

In keeping with modern practice, the author points out that the device is best employed if the output is amplified and caused to move the core of a similar unit in order to produce an equal and opposite compensating signal. The closed-loop servo, so produced, is relatively unaffected by supply voltage variations and other variables. In practical form, two primaries and two secondaries are provided and these can be interconnected in several ways. The author gives circuits for various applications, including those for performing simple mathematical operations such as addition, subtraction, and multiplication. Range adjustment is accomplished electrically and between wide limits.

The device can be used as an inductance balance, a much older principle, but its distinctive advantages lie in the use of the transformer principle. Herein lie countless possibilities for automatic methods for weighing, density, magnetic susceptibility, surface tension, or any phenomenon in which small forces are to be measured and recorded. The system exerts no restoring force on the moving core element.

In this connection it may be advisable to point out the existence of much older devices which depend upon a change in the air gap of a differential transformer. Some of these are extremely sensitive and applicable to a wide range of problems. They are thoroughly described in the monograph by Batcher and Moulic (*2*).

ELECTRONICS

It is almost impossible to review progress in this field for the special purpose of this survey, because the voluminous contributions are more properly classified under function and in this category one may list contributions to every known physical quantity—mass, length, time, pressure, etc.—as well as derived functions such as velocity, acceleration, discrimination, and simultaneity. In this respect, it is to be hoped that there will soon be more compilations like Marcus and Zeluf's collection of circuits which are grouped more or less under function (*21*). Such classification would afford the investigator a wide choice of circuits for almost every conceivable problem. This in effect would say, "You name it, and we will tell you how to do it." The few examples cited below may be of interest to the analyst, because they are concerned with problems similar to those encountered in well known analytical instruments.

The use of multigrid tubes as electrometers is discussed by Prescott (*32*), who shows that the 6BE6 and 7A8 are suitable and capable of operation with grid currents as low as 10^{-13} to 10^{-14} ampere. Transconductances of the order of 5 μa. per volt and an amplification factor of 300 are attained. This article includes valuable hints about cleaning and coating in order to obtain good insulation. A more detailed exposition of methods for reducing noise and leakage is given by Glass (*11*), in which choice of materials, method of construction, cleaning, and impregnating techniques are evaluated.

A device which has been used for some time in sound recording is now generally available and will find many additional uses. This is the R.C.A. 5734 tube, a mechano-electronic transducer. This is a triode transducer intended to provide a method of translating mechanical vibrations into current variations. It is based upon the principle of mechanically moving the plate of a triode with respect to a fixed grid and thus changing the plate current.

In this tube the plate shaft extends through the center of a thin metal diaphragm. The deflection sensitivity is 40 volts per degree deflection of the plate shaft. Vibrations up to 12,000 cycles per second can be measured. Earlier versions of this principle were proposed for static measurements of minute displacements, of the order of microinches, and presumably this tube can be used in similar fashion under the correct conditions. A few of the more important characteristics are: amplification factor 20; plate resistance 72,000 ohms; transconductance 275 microhms; moment of inertia of the plate 34 mg. cm.^{2}; maximum permissible displacement of plate shaft 0.5°. The 5734 tube weighs 1.5 ounces (1.75 grams) and has a length of $1^9/_{32}$

inches and a diameter of $^5/_{16}$ inch. It can be mounted in any position by means of a supporting clamp. Further details are available in the Radio Corporation of American data sheets on this tube and in two publications by Olson (*28, 29*).

Constant current regulators are useful or mandatory in many applications and a new approach is afforded by the work of Minett, MacRae, and Townsend (*22*). They derive a corrective error signal by balancing the *RI* drop across an incandescent lamp (carrying the load) against a VR-90 voltage regulator tube. Inasmuch as the lamp burns at high temperature and the losses are largely radiative, the effects of ambient temperature are negligible. They achieved a regulation at 250 μa. through a 25-watt lamp of 2 to 3 parts in 10,000 over periods of hours. The art of electronic current or voltage regulation is in a highly developed state and the various techniques are summarized in several reference texts (*13*). Despite this, there is unremitting search for improved circuits and reference elements, of which this paper is a good example.

Further advances in the electronic art are best illustrated in some of the remaining discussion.

SERVOMECHANISMS

Under this heading it may be useful to describe some components as well as several experimental techniques which illustrate the increasing trend to employ the servomechanism principle.

The magnetic fluid clutch, first described by Rabinow (*33*), has been made the basis of a fast-acting servo and is described by Bettis and Mann (*4*). An interesting alternative to this clutch is the Johnson Rahbek clutch made by Metropolitan-Vickers Electrical Co., Ltd. It consists of a polished disk, of high resistance semiconductor, and a polished metal disk, both flat to 10^{-4} cm., which are pressed lightly together. When a potential is applied between them, a force of attraction is developed which is approximately proportional to the square of the potential across the disks. A small clutch operating on this principle has been exhibited which transmits a torque of several hundred gram-centimeters with a negligible power consumption. No further information is available to the author; it has been found in a report by Rymer (*37*) describing apparatus shown at the Physical Society's exhibition.

An extremely useful and compact device is the Metron series of speed changers (Metron Instrument Co., 432 Lincoln St., Denver 9, Colo.). These are 6-ounce in-line speed reducers of both fixed and variable speed ratios. Ratios of 36 to 1 are available with 70 ounce-inch or 0.025 hp. torque rating. They exhibit maximum torque at zero speed and can tolerate speeds as high as 20,000 r.p.m.

It is often necessary to decide upon a method for presenting the variables to a servosystem. In an extensive standardization program, a means for controlling the rate of change of temperature was required. Although program controllers are often called upon to do just this, Bigg and Jones (*5*) have evolved a unique and fundamental approach. They have shown that the temperature rate of change may be controlled automatically by transforming it into a temperature difference through the agency of two elements of differing thermal time constants. The temperature difference is used to control the heat input or extraction. The temperature difference is measured by thermocouples and applied to a mirror galvanometer. The latter controls phototube relays which operate heating and cooling units, respectively.

Simple on and off regulation of a system results in oscillation about the desired control point and only the simplest kind of system is properly regulated in this manner. Most servosystems require a feed-back signal proportional not only to amplitude but also to rate. Herscher (*17*) has described a device which avoids slide wire and commutator troubles by having a servo motor drive an air capacitor to produce a rate-sensitive signal. The capacitor is in a tuned-plate, tuned-grid oscillator circuit and changes the plate current on either side of the resonance peak.

A good example of the application of electrical concepts to a distinctly hydraulic problem is Hill's design of a liquid metering system for small rates of flow (*19*). His system admits liquid to an adsorption column, and is constant in flow rate to 0.2% over a 30 to 1 range of flow. A pump is driven intermittently by an electronic timing system. A smoothing network of flexible rubber tubing with intermediate sections of glass capillary tubing produces uniform flow. This is the hydraulic analog of resistor-capacitor networks for filtering direct current supplies.

A very elegant and ingenious application of the servo principle is Mitchell's method of measuring small forces (*23*), which was developed to measure the yield value of non-Newtonian liquids and to determine the viscosity of small samples.

In either case the force is appropriately applied to the pointer of a milliammeter and the current in the meter coil is adjusted until exact balance is restored. The exact value of current is read on another series-connected milliammeter. Although this could be done manually, it is more neatly accomplished by mounting a vane on the pointer assembly which controls a 455-kc. oscillator. Output of the latter automatically rebalances the meter and the compensating current can be recorded. The author indicates that although care must be taken in protecting the jewel bearings, no difficulty is experienced with movements of 30-ma. sensitivity or greater. An estimate of the sensitivity of this technique may be inferred from the measurements of viscosity. In this application, the liquid sample is rotated in a thermostated cup in which a small cylinder is suspended. The torque exerted upon this cylinder by the liquid is communicated to the meter system and is automatically balanced as described above. In the case of water, the current required for rebalance is 30 microamperes.

RESEARCH IN INSTRUMENTATION

For the reasons mentioned in the beginning of this review, it is becoming easier for the investigator to devise new experimental methods and techniques. It is legitimate to ask why the investigator should wish to engage in instrumental research, when there are so many instrument companies far better equipped to do so. There are several good reasons.

There are many types of measurement for which there are no suitable instruments. The instrument manufacturer cannot be expected to do the necessary research and development unless he can be assured that there will be a reasonably widespread demand for the instrument. Many standard instruments need extensive modification in order to solve a special problem, and this is often best done in the laboratory where the special requirements are best understood and where design features can be changed at a moment's notice. A third reason, which is a good excuse, is an interest in instrumentation and the conviction that instrumental research and progress are important.

Fortunately, it is no longer absolutely necessary to have access to a first-class machine shop, at least in the earlier stages of instrumental research. Electronic components in the form of stabilized power supplies, alternating and direct current amplifiers, and measuring equipment of all kinds are readily available. Well designed "bread-boards" (Kepco Laboratories, Inc., 149–14 41st Ave., Flushing, L. I., N. Y.) can be purchased in which new circuits can be set up in a few moments. Finally, it is possible to purchase compact and skillfully packaged units of the plug-in variety, so that when one has established the circuit requirements these can be incorporated in a trim, professional, and essentially foolproof form. Some of these units are illustrated below.

With the advent of precision mechanical components which are carefully standardized, one may set up fairly elaborate mechanical systems to be used in conjunction with electronic and accessory equipment such as recorders and controllers. A brief description of these components has been given (*26*) and some of the following applications illustrate typical uses.

The base plates, legs, mounting brackets, ball bearing pedestals, gears, and shafting are available in great variety from Servomechanisms, Inc., Old Country and Glen Cove Roads, Mineola, N. Y. No tools other than two small Allen-head wrenches are required for any assembly. A profitable feature for the small laboratory is the fact that an instrument assembly can be dismantled in a few minutes after it has served its purpose; the parts can be returned to storage awaiting another use.

Figure 2 shows all the essential parts for a recording polarograph as set up in the author's laboratory.

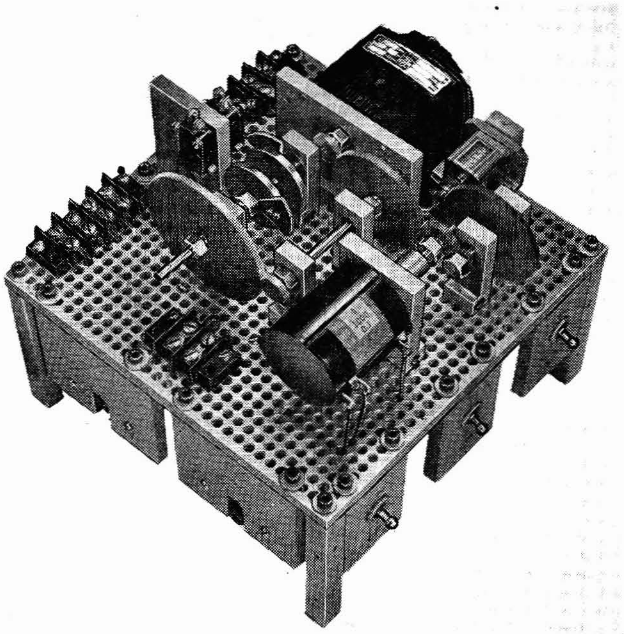

Figure 2. Polarographic Scanner Assembled from Standard Mechanical Components

A synchronous motor is mounted on a standard pedestal block and coupled through the precision stainless-steel gear train to a Helipot and also to a counter which indicates the applied potential directly. Two cams with associated microswitches are preset to prevent overdrive by the motor. Pedestals mounted below the table accommodate line and reversing switches, potentiometers, and rheostats for span adjustment and sensitivity control. One drawback, in comparison with more elegant polarographs, is the omission of any arrangement for quick return to zero voltage. In this case, the drive is reversed at the same speed and unless the analyst is busy preparing another sample, this time is wasted. The output is connected to a standard 0 to 2.5-mv. Brown Electronik recorder.

Typical polarograms with negligible damping and high damping are shown in Figures 3 and 4. It requires about 1 hour to reassemble this instrument, including the simple wiring. When only an occasional polarogram is required, this almost justifies the use of the parts, for other more pressing uses. Conversely, the total cost of keeping the assembly intact is small.

The scanner element of a reflectance densitometer is shown in Figure 5.

A special aluminum drum with steel tape scale is mounted on a shaft geared to a synchronous motor. An integral scanning unit is shown in the foreground containing a lamp, lens, filter holder, and viewing objective which focuses a small image into a phototube. Special vacuum phototubes, including carrier frequency-modulated types or small barrier layer cells, can be used. The photocurrent is registered on an electronic potentiometer. By providing a plastic drum or concentrically mounted glass cylinder, and making the necessary changes in the optical train, samples can be measured by transmittance. This device is used primarily for the automatic examination of paper chromatograms, and that use is discussed elsewhere in this issue (*6*). An example of its use in recording a print of the iron spectrum, by reflectance, is shown in Figure 6.

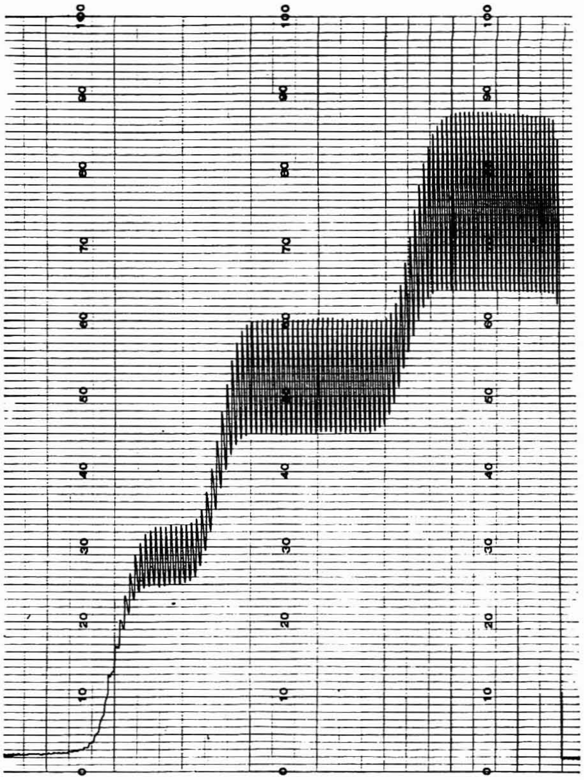

Figure 3. Polarogram with Negligible Damping

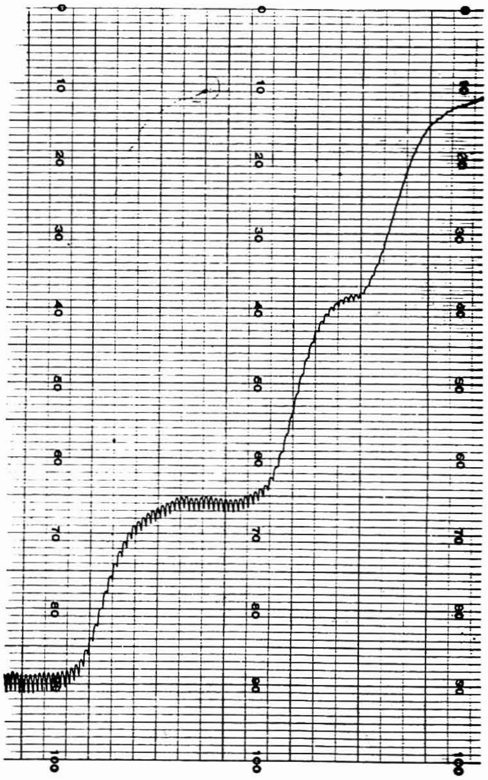

Figure 4. Polarogram with High Damping

A more elaborate installation is shown in Figure 7, which was designed and assembled by Servomechanisms, Inc., to the author's specification. Although the entire assembly is very compact, it retains a complete degree of flexibility, in that every element is a separate standard unit which can be unplugged, removed, or replaced in a matter of 10 seconds. It is a general-purpose servosystem which by a simple change in connections can be used as a self-balancing alternating current Wheat-

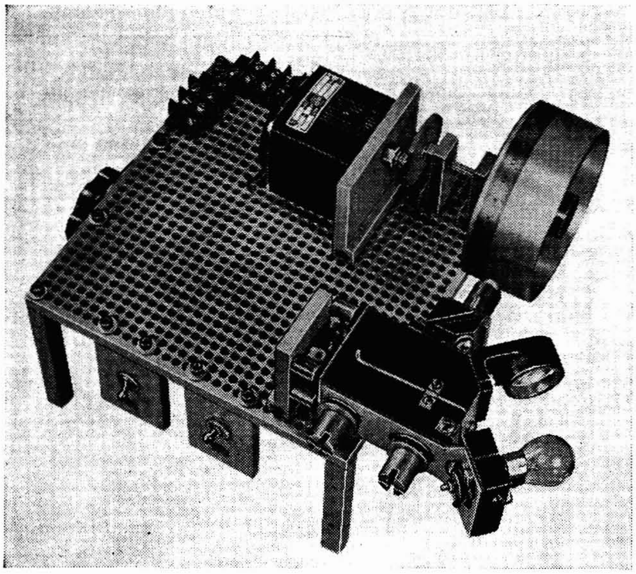

Figure 5. Reflectance Densitometer

stone bridge or self-balancing direct current potentiometer. For both uses, the output appears as a shaft rotation proportional to resistance change in the bridge or to input potential in the potentiometer. In addition, a first derivative signal is available in the form of a direct current voltage, proportional in magnitude to the rate of change of the primary variable and of polarity indicative of the direction of change.

In Figure 7 two of the snap-on dust covers have been removed to show the typical compact arrangement of components. At the upper left is a stabilized power supply and next on the right (uncovered) is the servoamplifier. To the right of this is the servo unit consisting of a Diehl two-phase motor geared to a continuously rotatable Fairchild balancing potentiometer and also to a direct current tachometer which generates the first derivative signal. This unit is assembled from the standard mechanical components. At the lower left of the main unit is a modulator, which converts small direct current potentials to alternating current for subsequent amplification. At its right is another unit which provides a very constant direct current reference potential for potentiometric applications. Each unit in this servosystem is interconnected with its companion units by subpanel plugs cabled together in a sturdy harness. For the various applications, requiring different interconnecting circuits, the entire cable harness is removed and quickly replaced by the appropriate cable. The usual panel light, main switch, fuse, and outlet connectors are visible in the lower right. The main panel is supported in sloping position by a chromium-plated steel tubing cradle.

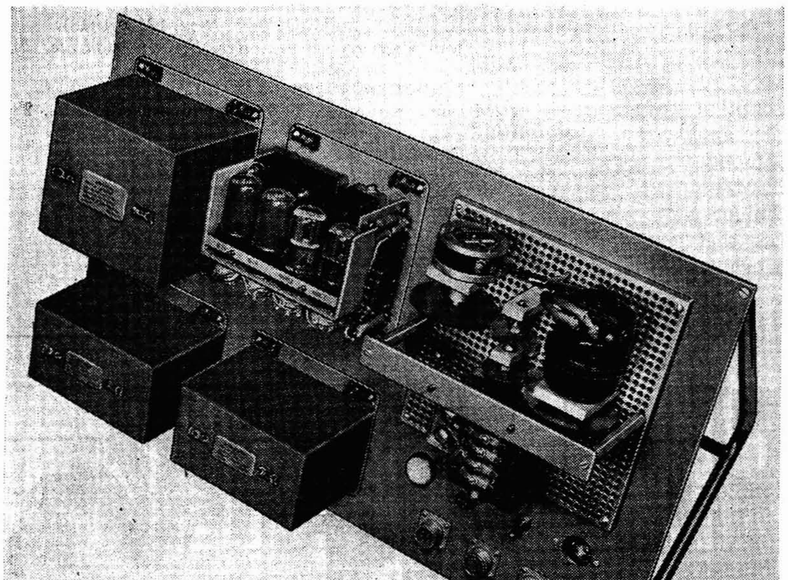

Figure 7. General-Purpose Servosystem

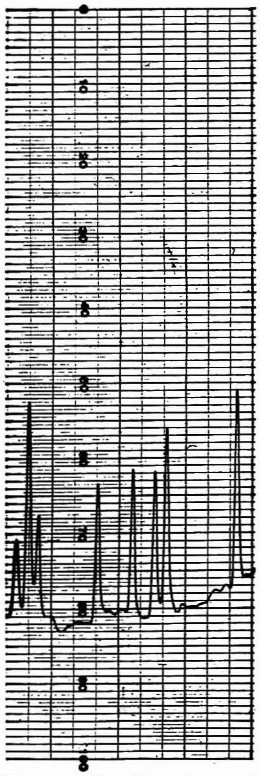

Figure 6. Portion of Iron Spectrum by Reflectance Densitometer

This servosystem possesses general utility in the research laboratory, for the relatively high torque shaft output responds almost instantly to a change in direct current input or resistance change in the bridge circuit and the rate-proportional signal is also useful. Consequently, any process which can be converted to a small direct current signal or resistance change can be measured or controlled with this system.

Figure 8, *A*, illustrates an interesting case in which an ordinary oxidation-reduction titration was recorded potentiometrically in the usual manner by a Brown Electronik recorder. When the same system was connected to the servomechanism, the output shaft rotated in exact proportion to the instantaneous value of

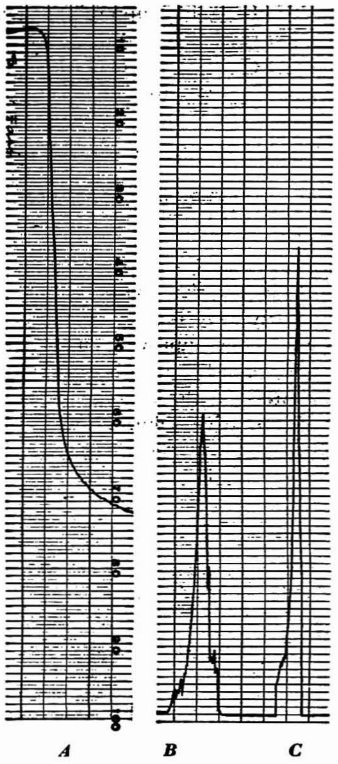

Figure 8. Potentiometric Titration Curves

A. Conventional curve
B. First derivative recording
C. First derivative recording, back-titration

e.m.f. of the electrode system. Of greater interest was to connect the output of the first derivative generator to the recorder. As seen in Figure 8, *B*, this draws the first derivative of the titration curve. Although the reagent was fed to the solution at a slow and uniform rate, the curve exhibits perceptible steps despite a well defined maximum. This was readily shown to be due to a mixing lag, since the oxidant was dilute potassium permanganate and minor inhomogeneities in mixing could be seen. Figure 8, *C*, shows the differential curve obtained when a relatively large increment of reductant was added in back-titration. Here the abrupt transition yields a perfectly smooth curve. It is evident that the servosystem is fast-acting and both shaft output and derivative signal impose no lags on the system greater than those inherently characteristic of the system.

The alternating current bridge arrangement of this servomechanism would seem to be very useful for automatic conductance titrations, although it has not been tried in this application. For any titration in which the conductance decreases before the end point, and rises after that region has been passed, a record of the first derivative signal would yield an essentially constant straight line. At the end point it would drop to zero, reverse polarity, and draw another essentially horizontal line proportional to the slope of the second part of the conductance curve. Electrically, this example is clear cut. In practice, it would require addition of reagent at uniform rate, uniform stirring, rapid attainment of equilibrium, and negligible temperature change (or compensation). Several titration reactions would meet these requirements and means are available for controlling these factors.

These few illustrations may serve to indicate what can be set up with readily available mechanical and electrical components. Perhaps some equally enterprising manufacturers will supply the investigator with readily assembled optical components, so that one may quickly set up the elements of an optical train. Here too, it should be possible to provide a universal bedplate, with easily adjustable supports for lenses, prisms, mirrors, slits, and light sources. Something more flexible than the conventional optical bench is needed. There are few instrumental problems which would be too difficult to handle, if versatile optical, electronic, and mechanical assemblies were available. It is therefore easy to appraise the author's initial statement that instrument design has almost reached a steady state wherein most of the necessary tools are at hand.

SUMMARY

As before, this review has intentionally avoided the description of many very important analytical instruments, some new and some highly improved, on the basis that they are more appropriately discussed in other reviews in this issue. What has been described in the way of new components, concepts, techniques, and tentative solutions may help to emphasize the fact that all instrumentation is enriched by the smaller, less spectacular advances. There is a growing realization that the fundamental concepts and practices of instrumentation are generally applicable in all branches of science and technology. In the sense implied by Condon, modern instrumentation seems to have all the earmarks of a distinct profession.

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INORGANIC MICROCHEMISTRY

PHILIP W. WEST
Louisiana State University, Baton Rouge, La.

THE scope of the present discussion is essentially the same as that established in the first review of inorganic microchemistry (*314*). Most of the references to developments in such fields as polarography, light microscopy, electron microscopy, fluorometry, nucleonics, and spectrometry are left for more detailed discussion in accompanying reviews, although strictly speaking, they represent true examples of important microchemical techniques. Likewise, most of the developments of applied microchemical methods are left to the reviews of analytical progress in the various industrial fields.

BOOKS AND REVIEWS

Progress in any field is greatly influenced by the appearance of authoritative books. A noteworthy contribution in the field of microchemistry is the recent appearance of the book "Specific, Selective and Sensitive Reactions" by Feigl (*72*). This book is unique in that it is an unusually complete record of the profound knowledge and experience of its author; it is not a mere abstract of collected papers but is a critical development of chemical theories based on intimate knowledge of the literature of analytical chemical researches. The book deals mainly with the chemistry of coordinated compounds, including such topics as complex ions and molecules, organic reagents, masking and demasking, induced and catalyzed reactions, surface effects, fluorescence, and photoreactions. No corresponding material has ever been presented in one place before and it seems safe to say that there is no one in the field of microchemistry who could not profit through the study of this volume.

A second significant publication is the third report of the Committee on New Reagents and Reactions of the International Union of Pure and Applied Chemistry (*126*). This report provides a broad survey of accepted tests for inorganic analysis based on the combined considerations of a number of international authorities in the field. A somewhat similar contribution has been made by Wenger and Duckert together with van Nieuwenburg and Gillis (*313*), all members of the committee. They revised the second report, adding a number of photomicrographs and some new tests. The book is a valuable reference, although being based on the second report it does not include tests published since 1943. A short manual by Ingram, Belcher, and Wilson has been published recently (*124*) which seems to fill a need for an introductory reference. The book "Aquametry" by Mitchell and Smith (*193*) contains many procedures that are microchemical in scale. Although this monograph deals solely with the determination of water (based mainly on the use of Karl Fischer reagent), there are so many important applications of such determinations that reference to this work should be of help to many microchemists.

Review articles have covered a wide variety of subjects. Wilson (*320*) has presented a thorough review of methods for determining molecular weights, including a number of techniques suitable for microchemical work. Electrometric methods applicable to water analysis have been reviewed by Janssen (*129*), and West has reviewed (*316*) important procedures used in the estimation of free chlorine in water. The applications of conductometric measurements in microanalysis have been summarized by Stock (*275*), and a group of seven papers covering various aspects of nucleonics (*8*) should be of general interest. Other reviews include those of Piquott (*231*), which is concerned with sulfur determinations in metallurgical products; Vanossi (*305*), which deals with the separation and estimation of tin; and Beaucourt (*18*), which covers gravimetric, titrimetric, and potentiometric methods for determining halogens.

A broad survey of absorptiometric methods of analysis together with a discussion of the construction of various absorptiometers has been published by Coumou (*48*). A review of various methods for determining trace elements in plants and animals has been given by Bertrand (*25*).

A general survey of the uses of organic reagents for inorganic analysis has been presented by Cattelain (*40*), while comprehensive reviews of the analytical chemical applications of 8-hydroxyquinoline (8-quinolinol) and benzidine have been written by Cimerman (*44*) and Horvorka (*121*), respectively.

APPARATUS

Microchemistry must depend often on sensitive measuring devices, and the introduction of new instruments is therefore of real interest. The description of a microbalance employing a radioactive detector seems very significant (*76*). The balance described is not only sensitive (1 microgram) but is unique in that it shows little change in sensitivity with varying load. The capacity per pan may be as high as 50 grams. A conventional balance for submicro work, which can be used for weighings involving differences of less than 1 microgram, has been described by Ingram (*125*).

The double-beam ultraviolet spectrophotometer of Kivenson, Osmar, and Jones (*137*) seems to possess many desirable features which would make it of value for many analytical applications involving absolute intensity determinations. The article by Stross (*284*) may be of interest to users of the Spekker absorptiometer.

Calibrations are always important, and the technique of Shead (*259*) for calibrating ocular micrometers is novel. The method depends on preparing metal beads whose diameters can be calculated from the density of the metal used and the weight of an individual bead as determined on an assay balance.

Stock and Fill have described a number of gadgets of use around a microchemical laboratory (*277–282*). Other items of interest are Lucite beakers for use with pH meters (*56*), a spray pipet for use in washing precipitates (*101*), and the refractive index comparator of Dollar (*58*) which permits changing of immersion liquids until match indexes are obtained for the crystal unknowns and the standard liquids. Nygaard has proposed a micromanipulator in the form of a screw-operated pipet which is attached to the objective of a microscope (*212*). Although intended primarily for biological work, this device has a number of potential uses in microchemical investigations.

A gas buret for use in measuring small volumes of gases has been devised by Burke (*32*), and Lewis has described a simplified Blacet-Leighton apparatus for the microanalysis of gases (*172*). The analysis of gases evolved from metals has been discussed by Keilholtz and Bergin (*136*), and Demidenko and Geller have described (*55*) a gas analyzer for use on 1 to 2 ml. of sample. Other pieces of equipment of possible interest are a melting point and microsublimation block (*215*), a vacuum still (*13*), an autoclave (*100*), and an electric muffle (*102*).

SEPARATIONS

Analytical separations are of extreme importance, and the introduction of efficient techniques for isolating desired components or removing unwanted interfering substances is of such value as to compare with the discovery of new reagents. In fact, the ultimate value of most reagents is dependent on the availability of suitable separation techniques. Two points might be emphasized in connection with this general topic. First, separations need not be time-consuming nuisances; many spot tests, for example, involve the use of masking agents for the elimination of interferences so that the offending material is sequestered by the mere addition of a drop of appropriate reagent. In colorimetric analysis, separation procedures may be used very effectively and where organic reagents are used, the extraction and color development for final analysis may be combined in such a way that the necessary separations are inherent in the method itself. The second point to be brought out is the difficulty of locating a given separation tool for a specific problem. Authors seldom emphasize complexing agents, extractants, gathering agents, etc., in the titles of their papers and as a consequence, abstracts do not include cross references to this vital phase of the analytical method and the analytical chemist must therefore rely on past experience and acquired arts gleaned from years of observant reading.

The preceding remarks are included for emphasis. The reviewer feels that one of the responsibilities of surveying the advances in inorganic microchemistry should be the collecting and organizing of significant procedures for separation.

Chromatography. One of the most active fields in analytical chemistry at the present time is that of chromatography. From the standpoint of inorganic microchemistry, paper chromatography is especially attractive. Lederer has used this technique for the separation and detection of chloride group anions (*171*); thiocyanate and iodide bands are located by treatment with ferric ion and hydrogen peroxide, while chloride and iodide are located by a silver nitrate treatment followed by a dilute nitric acid wash and exposure to hydrogen sulfide. Lederer has also described a method of separating silver, gold, platinum, palladium, and copper using a paper cylinder as the absorbent and a mixture of butanol and hydrochloric acid as the developer (*170*). He has also proposed the separation of antimony on paper strips, using dilute hydrochloric acid as the developer (*168*), and the separation of anions on paper using butanol saturated with aqueous 1.5 N ammonium hydroxide (*169*). Lacourt and collaborators separated a number of inorganic impurities from various organic solvents using paper as the absorbing material (*161*). Pollard and co-workers combined paper chromatography with fluorometric methods; twenty-four different cations were separated on paper and sprayed with organic reagents, and the bands were located by observation under ultraviolet radiation (*232*). Burstall and co-workers studied the separation of a number of metals on paper using methyl propyl ketone containing 30% hydrochloric acid (*33*); they also employed cellulose pulp for constructing columns, which introduces an interesting innovation on the standard practices.

The comprehensive paper by Arden *et al.* (*11*) describes the separation of many inorganic ions on paper, together with methods for subsequent determination.

No discussion of paper chromatography would give a true perspective if the work of Müller and Clegg were overlooked. These investigators have shown how instrumentation can be introduced for following the development of paper chromatogram—paraffin barriers are used to channel the solution and optical methods are employed for recording band positions (*197*).

In addition to paper chromatography, the use of alumina columns finds numerous applications for the separation of inorganic substances. Croatto has used such columns for separating rare earths (*49*), and Sacconi has given data pertaining to general separations of inorganic ions on alumina (*249*). A significant innovation is that of Meinhard and Hall (*185*), who propose the use of "surface chromatography"; this employs powdered adsorbents fixed to microscope slides by means of suitable binders. The technique corresponds to capillary separations on paper but offers more flexibility.

Complexation. Complexation probably offers the nicest and most generally applicable method for masking interfering ions. Particularly in the case of colorless inorganic complexes, most of the published work dealing with the coordination compounds formed are aimed at the elucidation of structure and the development of theory rather than the practical application of such substances. Knowledge of various complexers is therefore limited and applications are more or less restricted to standard inorganic complexers such as the phosphates and halides, cyanides, thiocyanates, thiosulfates, and a few chelating agents such as oxalates, citrates, and tartrates. It is to be hoped that more classified information will be published in the future by analytical chemists.

The stability of metal complexes has been discussed by Irving and Williams (*128*), who conclude that stability increases with the electronegativity of the metal involved. An interesting article dealing with molybdate and tungstate complexes (*204*) describes the use of citrates to mask tungstic and molybdic acids to permit determinations of sulfate and chloride. Chenery (*42*) has used thioglycolic acid as an inhibitor of iron interference in the colorimetric determination of aluminum. The masking of molybdenum, tungsten, and vanadium by means of fluorides has been discussed by Feigl (*70, 71*); demasking can be accomplished by addition of boric acid, which results in the formation of the very stable tetrafluoroborate complex. Phosphoric acid has been used by Pieters, Hanssen, and Geurts to eliminate the interference of iron in the hydrogen peroxide method for the determination of chromium (*229*). Cyanides have been used by Bourson and Fayette to complex heavy metals which interfere in the Titan yellow method for the determination of magnesium (*29*), and Šícha has recommended the use of cyanide, together with tartrate, for use in sequestering metals likely to interfere in the determination of aluminum in steel (*269*).

Various chelating agents have been employed for separations in addition to the thioglycolic acid, mentioned previously. Citrates have been used to prevent interferences due to tungsten, tantalum, and niobium (columbium) in the colorimetric determination of tin (*203*), and nickel has been determined by means of dimethylglyoxime, with the use of citrates as a conditioner to prevent iron interference (*4*). Tartrates have been employed by Busev and Korets (*35*) to prevent antimony interference in the thiourea method for the determination of bismuth. Lur'e and Ginzburg (*176*) suggest the use of fluorides or tartrates as a means of eliminating antimony interference in the iodide method for determining bismuth. Good examples of the value of seldom used complexers are the recent applications of malonic acid. Willard, Mosher, and Boyle employed an acetate–malonic acid conditioner in the determination of copper by means of dithiooxamide (*319*). West and Compere used malonic acid to prevent interferences due to iron, nickel, cobalt, and manganese in the colorimetric determination of copper in water by means of the same reagent (*315*).

Ammine and ammine-type complexes are often used in analytical work. Laitinen, Onstott, Bailar, and Swann studied the behavior of copper complexes of ethylenediamine, propylenediamine, diethylenetriamine, and glycine (*165*). Souchay and Faucherrie have used salts of ethylenediaminetetraacetic acid as supporting electrolytes (*272*); the complexes formed in such solutions are so stable that few metals are reduced at the dropping mercury electrode and as a consequence cobalt can be determined in the presence of excesses of most other metals without separations other than those inherent in the use of such complexing bases. Nelson and Gantz (*205*) have studied the relative stabilities of a number of copper complexes.

Extraction. The use of extraction procedures for isolating substances for analysis or for removing interfering materials is not as widely practiced as the method justifies; in many instances very sharp separations can be secured and a wide variety of partitions are possible because of the diversity of solvent types available and the multitude of organic reagents that can be utilized as extraction aids. Current examples of extraction methods give an idea of the scope of the possible applications.

McBryde and Yoe (*178*) have proposed a method for the determination of gold as the tetrabromoaurate. The highly colored complex can be extracted into isopropyl ether and thus separated from possible interferences. The nitrite ion can be isolated as isoamyl nitrite by extraction with carbon tetrachloride and isoamyl alcohol according to Ubaldini and Guerrieri (*300*), and the nitrite then determined colorimetrically.

The determination of trace constituents in the presence of large amounts of iron can be accomplished in many cases by extracting the iron as ferric chloride, using amyl acetate as the extractant. Wells and Hunter claim that the amyl acetate is a more efficient solvent than either diethyl ether or isopropyl ether (*312*). Robinson has used isopropyl ether to extract the colored complex of molybdenum and thiocyanate (*243*) in the colorimetric determination of molybdenum in phosphate rock, and Piper and Beckwith have determined small amounts of molybdenum in plants by extracting the cupferron complex with chloroform (*230*), and precipitating the metal from the extractant by means of toluene-3,4-dithiol; the precipitate can then be dissolved and a photometric estimate of concentration made without fear of interference. Kuskova has shown that aluminum can be extracted as the oxinate (*153*), and Abrahamczik (*1*) has determined magnesium with Titan yellow by first removing iron, aluminum, manganese, copper, vanadium, and uranium by complexing them with acetyl acetone and extracting the chelates into a mixture of carbon tetrachloride and acetyl acetone.

Electrodeposition. The use of electrodeposition provides many useful separations, and the convenience of the mercury cathode makes the method especially attractive. Silverman has utilized the mercury cathode and electrolyzed high-temperature alloys to remove interfering metals in the determination of aluminum and titanium (*271*), and Cooper and Winter have used the same general method for separating interfering metals in the colorimetric determination of vanadium in steels (*47*). Wiberley and Bassett have electrolyzed steel samples in water-jacketed mercury cathodes until iron-free. Aluminum was then precipitated with 8-hydroxyquinoline for subsequent determination (*318*).

Precipitation. Precipitation will always be a dependable method for use in making analytical separations, although it suffers a serious handicap for some work through the complications and time losses which occur because of the required filtrations and washings. A very important aspect of precipitations is the use of "gathering agents" for concentrating minute amounts of material for trace analysis. Because the determination of trace materials is becoming increasingly important, this phase of precipitation reactions is certain to arouse more and more interest.

Current examples of gathering agents and their application include the collection of traces of beryllium as the phosphate with aluminum phosphate as the gathering agent, described by Aldridge and Liddell (*5*). Ballard and Ballard have studied methods for the determination of trace amounts of bismuth in lead (*16*), and recommend the concentrating of the bismuth by coprecipitating it with ferric hydroxide. Kühnel-Hagen Hofman-Bang, and Gjertsen suggest the separation of traces of tin from iron, copper, and lead by collecting the tin on hydrated manganic oxide (*149*).

Organic reagents provide useful precipitations for separating and concentrating inorganic ions. Mitchell and Scott have used 8-hydroxyquinoline for concentrating metals prior to spectrographic examination (*194*) and have also studied tannic acid and thionalide for such work.

ORGANIC REAGENTS

The applications of organic reagents are covered more specifically under separate divisions of this review. This subject is so important, however, that some comment is necessary concerning general contributions to the theory and use of such compounds.

Feigl and Baumfeld have introduced studies of reactions which take place between fused 8-hydroxyquinoline and metals (*73*). These studies can easily lead to advances in theory as well as new applications of organic reagents. Korenman has discussed reagents reacting with nickel, ferrous ion, and palladium and has considered the structure of the reaction products (*141*). He has also considered organic reagents that react with boric acid and has applied theoretical principles in accounting for the behavior of various configurations (*142*).

A very interesting paper by Kuznetsov compares the reactions of a number of organic compounds and functional groups with analogous inorganic reactions (*157*). For example, enolic OH formation is compared to hydrolysis of inorganic compounds and the reactions of mercaptans are likened to certain sulfide reactions. The article is worth translating from the Russian.

Bobtelsky and Spiegler have studied the Vogel reaction (the formation of blue colors by adding alcohols to cobalt thiocyanate complexes) and conclude that the ratio of cobalt to chloride, bromide, iodide, or thiocyanate is 1 to 2 in some cases and that a coordination number of 4 is encountered in other situations (*27*). The effects of temperature and dehydrating agents on the blue color are discussed on the basis of the covalent structure of the complexes.

The reactions of tannin, especially the bismuth and cerium complexes, have been discussed by Holness (*115*). Holness and Pate have studied the precipitation of silica by tannin (*116*) and conclude that the reaction is not suited for the complete removal of silica in a single step.

Mallik and Mazumdar have studied 5,6-benzoquinaldic acid and found that it precipitates practically all bivalent metals (*180*). The reagent holds promise for the determination of copper, and may be of value in the determination of iron, inasmuch as the red precipitate formed with ferrous iron can be dissolved in cyanide solutions.

BIOASSAY

Bioassay methods often provide very sensitive means for determining inorganic compounds, and in many cases a high degree of selectivity is shown. Cuthbertson has discussed recent developments in bioassay methods (*51*). Gerretsen has considered methods for using *Aspergillus niger* in the estimation of plant nutrients in the soil (*92*), and Mulder has employed the same organism for the estimation of magnesium, copper, and molybdenum in plant tissues and soils (*198*).

GRAVIMETRIC ANALYSIS

Šícha has employed 8-hydroxyquinoline for the gravimetric determination of aluminum in steel (*269*), using tartaric acid and potassium cyanide as masking agents. Feigl and Baumfeld have used 8-hydroxyquinoline for the detection and gravimetric determination of thallium (*74*); water-insoluble inner-complex salts are formed with thallium (III).

The use of 1,10-phenanthroline for the gravimetric determination of palladium has been described by Ryan and Fainer (*247*). The precipitation is performed in dilute hydrochloric acid solutions and the precipitate is dried at 110° C. Other platinum group metals do not precipitate under the conditions described but do tend to cause slightly high results—indicating a tendency toward coprecipitation which is rather unusual in the precipitation of inorganic ions with organic reagents. Haines and Ryan have studied the determination of rhodium and recommend the use of 2-mercaptobenzoxazole or 2-mercaptobenzothiazole (*109*). Precipitation is from acetic acid solutions and the precipitates can be dried at 110° C.

Trujillo suggests the use of α-benzoinoxime for the determination of copper in pharmaceuticals (*297*). The procedure used is essentially standard.

Carbon in carbonates, cyanides, and alkali or alkali earth organic salts can be determined on 5- to 20-mg. samples by a combustion procedure which depends on final adsorption of liberated carbon dioxide on Ascarite (*164*). The determination of small amounts of carbon monoxide in air can be accomplished by the catalytic oxidation of carbon monoxide by passing it over platinized glass (*37*); the carbon dioxide formed is adsorbed on Ascarite. According to Stragand and Safford, sulfur in organic compounds can be determined by burning the sample in an atmosphere of oxygen in the presence of a platinum catalyst (*283*). The sulfur trioxide formed is adsorbed by a silver gauze with the quantitative formation of silver sulfate.

Bertiaux and Théry have found (*24*) that bismuth can be determined in the presence of large amounts of lead by use of potassium bromate. This procedure eliminates the use of prior separations of lead as the sulfate, and so eliminates error due to the loss of small amounts of bismuth which usually occurs during the lead separations.

Selenium has been determined in steel by a conventional precipitation using sulfur dioxide (*287*). A method for determining small amounts of water in nitrogen tetroxide has been described by Whitnack and Holford (*317*), and Pennington has devised a method for determining small amounts of water in Freon 12 using phosphorus pentoxide as the absorbent (*224*).

TITRIMETRIC ANALYSIS

A number of interesting titrimetric procedures have been proposed recently. Potassium has been determined by Bourdon (*28*) based on the precipitation of sodium potassium cobaltinitrite. The nitrite is then oxidized with an excess of potassium dichromate and the excess determined by titration with standard ferrous sulfate. Korenman and Gutnik have determined small amounts (50 to 70 micrograms) of calcium by means of an oxalate precipitation with subsequent permanganate titration (*144*), and Korenman and Glazunova have determined lead by precipitating it as the chromate (*143*); the chromate is then titrated iodometrically.

Freeman and McNabb have determined arsenic by precipitating it with hypophosphorous acid. The elemental arsenic is then treated with Koppeschaar's bromide-bromate solution so as to permit an iodometric determination (*85*); the method can be applied in the presence of antimony, tin, bismuth, and lead.

Titanium has been determined in steels by amalgam treatment and subsequent titration with ferric chloride, using thiocyanate as an indicator (*322*).

Gold has been determined by collecting with copper sulfide, removing the copper, and titrating with standard hydroquinone in the presence of *o*-dianisidine. This method, described by Milazzo (*189*, *190*), can be applied in the presence of palladium and rhodium but not iridium. Platinum interferes slightly owing to its color.

The cyanide titration for nickel has been applied by Generozov (*91*) to small amounts of nickel to steel.

Bismuth has been determined by Shchigol (*257*) by precipitating it either as the iodate or the chromate, and determining the excess precipitant by iodometric titration.

Gaillard and Gayte have studied the determination of chromium (*87*), and Generozov has presented procedures for determining chromium and vanadium (*90*).

Zinc has been determined by Cruikshank (*50*) by use of a dithizone extraction followed by treatment with ferricyanide solution and titration with dilute standard sodium thiosulfate solution. Copper has been determined (*285*) by means of a dithizone titration, and through the application of a modified iodometric procedure (*57*).

A titrimetric determination of boron has been described by Abramson and Kahane (*2*). The method is adopted from the usual boric acid titration in the presence of mannitol. The determination of phosphorus has been performed by molybdate precipitation followed by sodium hydroxide titration (*266*).

Sulfur has been determined by Pepkowitz (*225*), using a distillation procedure in which hydrogen sulfide was passed into an excess of standard hypochlorite solution. The excess hypochlorite was then determined iodometrically. Morris, Lacombe, and Lane have proposed that elemental sulfur be determined by means of the reaction between sulfur and sulfite to form thiosulfate (*196*). Excess sulfite added for the reaction is masked with formaldehyde, iodate is added, and the excess iodate is determined iodometrically.

Titrimetric methods for the determination of boron and copper have been presented by Scharrer (*252*).

Iodide has been determined in organic compounds by means of an iodometric titration (*104*), and fluorides can be determined satisfactorily using a new indicator, Chrome Azurol S (*191*). The iodometric determination of cyanide has been discussed by Lur'e and Nikolaeva (*177*).

Lingane and Pecsok have developed a titrimetric method for the determination of nitrate based on the reduction of the nitrate to form ammonia, using chromous ion for the reduction (*173*).

SPOT TESTS

The interest in spot test methods is attested to by the large number of publications that have dealt with this technique during the past year. From an unprejudiced (almost) point of view it can be said that these methods approach the ideal for practical qualitative analysis, and new developments should be watched with interest, for the full potentialities of the method have not by any stretch of the imagination been attained.

Pavolini and Gambarin have found that thiobarbituric acid can be used to detect copper at a dilution of 1 part in 20,000,000 and silver can be detected at dilutions of 1 part in 5,000,000 (*222*). These same investigators have studied *p*-dimethylaminobenzilidenethiobarbituric acid and believe that it is of value for the detection of silver and palladium (*221*). Silver and mercury can be detected by use of pinacyanol iodide (*306*); a blue color is produced by as little as 0.01 microgram of either silver or mercuric ions. Kul'berg and Ledneva have used formazylcarboxylic acid for the detection of silver, the limit of identification being 2.3 mg. at a dilution of 1 part in 136,000 (*151*).

Gold has been detected by means of 1-naphthylamine (*234*), and Tananaev has discussed methods for detecting platinum, palladium, iridium, rhodium, and gold in precious alloys; the tests are run on material dissolved from the surface of the sample by means of aqua regia and standard spot test procedures (*286*) are used for the analysis.

Iron has been detected by Scheil through the use of Ferroxyl paper (*253*); free iron can be located on stainless steel surfaces, for example, by pressing the moistened test paper against the metal surface and allowing 5 minutes for the reaction time. Manganese can be detected in aluminum alloys by bismuthate oxidation, and chromium can be detected through use of diphenylcarbazide (*21*).

The detection of aluminum by use of Aluminon has been modified so as to make the test specific, according to van Nieuwenburg and Uitenbroek (*207*). Sulfurous acid is used to prevent interference of chromium, indium, gallium, and titanium, while ethyl alcohol plus hydrochloric acid is used to prevent beryllium, scandium, zirconium, and iron interferences.

Peltier, Duval, and Duval have applied the various tests for nickel published during the period 1937–1947 and have concluded that Nioxime (1,2-cyclohexanedionedioxime) is the most satisfactory reagent available (*223*). Duval and Duval have made a critical survey of tests for cobalt (*64*).

Semiquantitative analysis for copper, chromium, manganese, and silicon, using spot test procedures, have been applied in sorting Duralumin, Chromansil, and Silumin alloys (*209*).

A new reagent for the detection of mercury has been proposed by Nazarenko (*202*). The reagent, 1,4-diaminodihydroxyquinone, is sensitive to 1 microgram of mercury at a dilution of 1 part in 1,000,000. Although the reagent must be made up immediately before use, it seems to hold promise because of its high selectivity. Gautier has suggested the use of the periodo derivative of methylene blue for the detection of mercury and tin (*88*), and methyl violet has been advocated by Kuznetsov (*158*) for the detection of tin(II); cuprous chloride gives a reaction similar to that given by tin and certain organic reducing agents interfere.

Phenothiazine has been studied by Duval (*63*) and its use as a reagent for silver, iron, and mercury is discussed.

The detection of vanadium has been studied by Hoste (*120*), who claims that a specific test is possible based on the use of diphenylbenzidine. A saturated solution of the reagent in glacial acetic acid gives a yellow color with small amounts of vanadium and a green precipitate forms at higher concentrations; the interference due to ferric iron can be masked by using fluorides. The limiting concentration for the test is 1 to 1,000,000.

A test for bismuth based on the reduction to the metal by means of formaldehyde has been described (*258*), and various spot tests of value in analyzing aluminum alloys have been described by Niessner (*206*).

The detection of uranium based on reactions with inorganic ions has been considered by Kohn (*139*), and a summary of various spot tests and microchemical tests has been published by Duval and Duval (*65*).

Hoste has studied diaminobenzidine and found it of value for the detection of vanadium and selenium (*119*). Its reaction with vanadium is similar to the benzidine reaction but, in the case of selenium, an intensely colored precipitate forms which permits the detection of this element at dilutions of 1 part in 1,000,000.

A survey of tests for rhenium has been made by Duval (*62*), and Kalugai has reviewed reagents suitable for the detection and separation of the alkali metals (*132*).

A very interesting paper by Okàč and Pech deals with the reactions of pyrogallol carboxylic acid and the alkaline earth metals (*213*). Calcium, strontium, and barium produce blue colors or blue precipitates with the reagent. There are a number of interferences with the test, but a color reaction for these metals is of real significance and further study along similar lines should be of interest.

Tellurium can be detected by means of anthraquinone-1-azo-4-dimethylaniline (*156*). The reaction takes place in strongly acidic solution and as little as 0.006 microgram of tellurium can be detected. Selenium does not react to give colors, but interferences do result in the presence of uranium, gold, iron, gallium, molybdenum, wolfram (tungsten), aluminum, bismuth, antimony, tin, mercury, and platinum.

Sulfur has been detected in organic compounds by sodium carbonate fusion in the presence of magnesium. Sulfur is thus obtained in the form of the sulfide. Liberated hydrogen sulfide is passed over lead acetate paper for final detection (*36*).

A number of standard tests have been described for the detection of fluorides (*38, 39, 182*).

Feigl and Feigl have proposed a new test for cyanide (*75*) based on the demasking of palladium complexes such as palladium dimethylglyoxime and palladium salicylaldoxime. A sensitive test for cyanide is suggested which can also apply for the detection of illuminating gas.

The detection of cyanide and ferrocyanide has been considered by Hubach (*122*), and Chao and Su have introduced *n*-chlorosuccinimide as a reagent for ferrocyanide (*41*). Tests for thiocya-

Table I. Colorimetric Determination

Ion	Reagent or Method	Reference
Aluminum	8-Hydroxyquinoline (8-quinolinol)	(*153, 159, 187, 318*)
	8-Hydroxy-7-iodo-5-quinolinesulfonic acid	(*52*)
	Miscellaneous	(*154*)
	Alizarin	(*261*)
	Aluminon	(*271*)
	Eriochrome-Cyanine R	(*292*)
Antimony	Starch KI	(*77*)
	Ascorbic acid	(*210*)
	Pyridine KI	(*203*)
	Rhodamine B	(*248*)
Arsenic	Hypophosphite	(*3*)
	Gutzeit	(*69*)
	Gutzeit	(*80*)
	Molybdenum blue	(*166*)
	Levvy	(*179*)
Beryllium	Naphthochrome Azurine 2B	(*5*)
Bismuth	Miscellaneous	(*16*)
	Thiourea	(*35*)
	Dithizone	(*167, 242*)
Boron	Turmeric	(*246*)
	Asurine blue S	(*295*)
Cadmium	Diphenylcarbazide	(*93*)
	Diethyldithiocarbamate	(*216*)
	Dithizone	(*264*)
Calcium	Picrolonic acid	(*138*)
	Pyrogallol–carboxylic acid	(*214*)
	Nickel nitrate + dimethylglyoxime	(*299*)
Chlorine	Starch + KI	(*60*)
	o-Tolidine	(*86, 181*)
	Tris (*p*-dimethylaminophenyl) methyl-tri-HCl	(*99*)
Chromium	Diphenylcarbazide	(*4, 229, 292*)
	Persulfate	(*10*)
	Periodate	(*162*)
Cobalt	Nitroso R salt	(*135*)
Columbium	Thiocyanate	(*7*)
	Molybdate	(*53*)
Copper	Ammonium citrate	(*97*)
	Phenolphthalein	(*103*)
	Diethyldithiocarbamate	(*133, 255, 262, 273*)
	Thiocyanate + acetone	(*195*)
	Dithio-oxamide	(*315, 319*)
Ferricyanide	Ferrous sulfate	(*152*)
Germanium	Molybdate	(*82*)
Gold	Hydrobromic acid	(*178*)
Indium	Dithizone	(*184*)
Iron	Thiocyanate	(*26, 107, 148*)
	Thiosalicylic acid	(*93*)
	1,2-Dihydroxybenzene-3,5-disulfonate	(*236*)
	Glycolic acid	(*260*)
	Isonitrosodimethyldihydroresorcinol	(*267*)
Lead	Molybdate + thiocyanate	(*66*)
	Tetramethyldiaminophenylmethane	(*93*)
	Hematoxylin	(*123*)
	Dithizone	(*239, 242, 255, 270*)
Magnesium	Titan yellow	(*1, 29, 226, 228*)
	Miscellaneous	(*150, 240*)
	Thiazole yellow	(*188*)
	p-Nitrobenzeneazoresorcinol	(*245*)
	Brilliant yellow	(*288*)
Manganese	Periodate	(*78, 162*)
	Peroxydisulfate	(*211*)
Mercury	Dithizone	(*127, 242*)
Molybdenum	Thiocyanate + stannous chloride	(*4, 243, 291*)
	Thiocyanate + sulfite	(*96*)
	Dithiol	(*230*)
Nickel	*o*-Nitrosalicylic acid	(*304*)
Nitrogen oxide	Diphenylamine	(*83*)
Nitrous oxide	Pyrogallate	(*254*)
Nitrate	Diphenylamine	(*174*)
Nitrite	Sulfanilic acid	(*300*)
Oxygen	Starch + iodide	(*227*)
Ozone	Indigosulfonic acid	(*59*)
Phosphorus	Molybdate	(*17, 46, 67, 108, 183, 251*)
	Strychnine-molybdate	(*22*)
Potassium	Cobaltinitrite	(*131*)
Rhenium	Thiocyanate + stannous chloride	(*186, 294*)
Silicon	Molybdate	(*113, 163, 199*)
Silver	Sugar	(*111*)
	Pyridine + persulfate	(*321*)
Sodium	Nickel uranyl acetate	(*298*)
Sulfur dioxide	Fuchsin	(*6*)
Sulfide	*p*-Aminodimethylaniline	(*84*)
	Lead acetate	(*308*)
Thorium	Oxalate + permanganate	(*241*)
Tin	1-Methyl-3,4-dimercaptobenzene	(*203*)
Titanium	Hydrogen peroxide	(*271*)
Tungsten	Thiocyanate + stannous chloride	(*43, 233*)
Vanadium	Phosphate + tungstate	(*47*)
	Oxine	(*301*)
	Ferrocyanide	(*309*)
Water	Tetraiodoplumbate	(*79*)
Zinc	Cacotheline	(*93*)
	Dithizone	(*192, 208, 237*)

nate have been developed by Kreshkov and Vil'borg based on reactions with potassium chlorate or ammonium molybdate (*147*). A test for hydrogen peroxide based on the reduction of ferricyanides has been suggested by Kohn (*140*).

COLORIMETRIC ANALYSIS

Although a separate review of colorimetric methods of analysis has been prepared, some mention of developments in this field must be made in the review of inorganic microchemical developments. The reactions used in spot test methods almost invariably find use in colorimetric work, and certainly most colorimetric procedures involve reactions that can be adopted for qualitative analysis. More important is the fact that well over 60% of the microchemical procedures involve colorimetry. Although no discussion of developments is justified here, Table I is included to summarize the applications of the various color reactions.

MISCELLANEOUS

A number of developments require review, which do not fit under the specific headings of the general discussion. For example, Urech, Muller, and Sulzberger have determined magnesium in aluminum alloys by distilling it at 800° C. and 0.001 to 0.0001 mm. of mercury (*302*); 0.2 to 10% magnesium can be determined accurately in this manner. Wehrli and Kanter have determined cyanides by evolving hydrocyanic acid which is isolated as Prussian blue. This is then treated with silver nitrate and the silver cyanide formed reduced to metallic silver; measurement of the diameter of the bead serves to establish the original cyanide concentration (*311*).

Korenman and Punchik have suggested the determination of cobalt by volumetric measurement of the bulk of potassium sodium cobaltinitrite precipitates (*145*).

Katz and Katzman have determined carbon monoxide in air over a range of 10 to 200 p.p.m. by means of thermometric measurements of the heat of reaction when the gas is catalytically oxidized (*134*); a catalyst of silver permanganate deposited on zinc oxide carrier is used. X-ray absorption methods have very interesting possibilities (*324*), and reference to the accompanying review should indicate the scope of such techniques.

Nephelometric methods have important applications in microchemical work. Bertiaux has determined small amounts of silver by converting it to silver perchlorate and measuring the turbidity (*23*). Geuer has determined sulfur in iron and steel by passing the gases of combustion into buffered lead acetate solutions; the lead sulfite formed can be estimated nephelometrically to within 0.3% of the truth (*94*). Potassium has been determined in biological and agricultural products by measuring the turbidity of the cobaltinitrite reaction product (*293*), and cadmium has been determined nephelometrically using the reaction between cadmium iodide complex and β-naphthoquinoline (*323*).

Determination of traces has been discussed in various sections of this review. A general survey of methods for the determination of trace elements in biological material is of interest (*117*), and the article by Parks and Lykken dealing with traces of aluminum is of value; aluminum can be separated by means of a chloroform-oxine extraction and the aluminum oxinate estimated using the polarograph or the ultraviolet spectrophotometer (*220*). An improved method for the determination of trace amounts of beryllium has been published by Sandell (*250*), based on the precipitation of beryllium and aluminum with ammonia in the presence of mercaptoacetic acid to keep iron in solution; final estimation of beryllium content is made fluorometrically with morin as the reagent.

Geilmann and Bode have advocated a boron test based on the formation and flame excitation of boron trifluoride (*89*). Because of the volatility and ease of excitation of this compound it is possible to run the test just outside of the Bunsen flame and so avoid interferences from calcium and sodium.

Goldstone has presented a systematic procedure for the isolation and detection of 26 common toxic substances sometimes found in food (*98*). Groupings such as "volatile," "metallic," "alkaloidal," and "nonalkaloidal" are used for the classification, and sensitive tests are given which are best suited for use in food analysis.

An interesting article by Fischer and Langhammer deals with microscopic identification of metals by refractive index measurements made on certain precipitates such as the oxinates, picrolonates, etc. (*81*). The precipitates are immersed in liquids of somewhat higher index and the mixture is then heated on a Kofler micromelting point apparatus. The temperature at which matching indexes are obtained serves to identify the sample.

The evaluation of accuracy of colorimetric methods is important; the article by Ayres is excellent (*12*) and the article by Hiskey should be consulted (*114*). The discussion by Benedetti-Pichler on precision weighings is also of general interest, the conclusion in this case being that the method of transposition of weights is to be recommended for exact work (*20*).

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INORGANIC GRAVIMETRIC ANALYSIS

F. E. BEAMISH

University of Toronto, Toronto, Canada

THIS review follows the arrangement adopted for the first issue (*6*), although in the section dealing with general procedures there has been some alteration of subheadings.

During the past year there has been an increased number of publications communicating progress of great interest to analytical chemists. Perhaps of primary importance are the researches dealing with the application of Amberlite resins to chemical separations. This field of investigation is only in the initial stages of development and much further knowledge of great value will be forthcoming in future years.

The work of Duval and his associates merits attention. These investigators have been making an organized effort to ascertain the dissociation temperatures of numerous precipitates used for gravimetric purposes.

GENERAL PROCEDURES

Preparation of Samples. Baryshev (*5*) discussed the experimental basis of a sampling method and the preparation of laboratory samples for analysis. Herteland (*57*) dealt with the preparation of samples of metals and alloys for analysis. He rejected the use of borings or shavings and recommended melting under prescribed conditions. With certain alloys this treatment resulted in a change in the proportions of constituents. This author (*57*) also discussed the losses incident to the formation of a mist during the dissolving of metals and alloys. The use of cover glasses did not eliminate the loss. In the analysis of clays, bauxite, feldspar, etc., Cadariu (*21*) suggested preliminary sintering with calcium carbonate. Tkachenko and Khripach (*131*) recommended drying samples of iron ore for 5 minutes at 150° to 160° C. in preference to drying at 105°.

Method of Selective Separations. Lur'e and Filippova (*82*) used cationites for absorption followed by selective extraction with alkali solution to separate antimony, molybdenum, tungsten, zinc, and aluminum from anions, also from elements that form basic hydroxides—e.g., iron and copper—and from arsenic in the arsenite form. By this procedure the coprecipitation incident to hydrolytic precipitation was avoided. Later the authors (*83*) reported the successful application of Wolfatit P for the separation of zinc and aluminum from iron, antimony and tin from arsenic, bismuth from copper or lead, and bismuth from antimony. Anion-exchange experiments were made with guanidine anionite. Chromate was absorbed and subsequently extracted with sodium hydroxide. Directions were given for the separation of chromate ion from nickel. Under specific conditions tin could be absorbed but not separated from antimony. Bismuth was efficiently separated from copper. In neutral or alkaline solution permanganate was reduced to manganese dioxide, absorbed, and subsequently extracted with sulfuric acid.

Kostrikin (*75*) stated that absorption by organolites was incomplete if a definite portion of the solution was mixed with a given amount of the sorbent. Filtering through a layer of the sorbent was preferable. Lur'e (*81*), dealing with small concentrations, rejected filtration through a layer of sorbent in favor of mixing the solution with the organolite followed by extraction. Osborn (*96*) recorded some analytical applications of *m*-nitrobenzoic acid to the separation of quadrivalent elements from the rare earth elements, etc. Excluding the interference of mercury and the possible interference of hydrolyzed tin salts, *m*-nitrobenzoic acid appeared to be a specific precipitating reagent for all quadrivalent ions except titanium. Ostroumov (*98*) discussed in considerable detail the methods of separation by pyridine, α-picoline, and hexamethylenetetramine. Pyridine was used as a regulator of the acidity of solutions during hydrolysis and for the formation of complexes. This work appears to be worthy of extended study.

Vanossi (*139*) published a review of his work on the separation and identification of elements which are distilled by acids. Included were the separation and identification of osmium, ruthenium, and germanium; and the identification of germanium, rhenium, and tin. Musante (*94*) examined the reactions between certain hydroxamic acids and metal ions with a view to analytical applications. The benzo derivative of hydroxamic acid was a sensitive reagent for copper and to a less degree for cobalt and nickel. Copper also formed insoluble anisohydroxamates. Gaspar y Arnal and Rojo (*46*) dealt with the applications of sulfites and sulfates to chemical analysis. They obtained quantitative separations of calcium and strontium, calcium and barium, and calcium and lead, and they (*43*) described the use of sulfites for separations of cupric ion from the ions of the alkaline earths and lead. Korenman (*74*) discussed relationships between the structure of various organic compounds and their ability to form colored and insoluble salts with nickel, ferrous iron, palladium, etc.

Preparation for Weighing. Duval (*31*) investigated the behavior toward heat of moist asbestos used in Gooch crucibles. Up to 73° a loss of weight due to water of imbibition was observed; from 73° to 283° the weight remained constant; at temperatures above 283° a regular loss of weight occurred. It was concluded that precipitates weighed in Gooch crucibles should not be dried above 283°. The temperature intervals producing constant weight of some 23 compounds used as precipitates were determined. The author (*29*) proposed to examine the optimum temperature limits for the ignition of about 600 substances encountered most frequently in gravimetric analysis. Shiokawa (*119*) determined suitable ignition temperatures for tannin precipitates of aluminum, beryllium, cerium, chromium, copper, iron, manganese, titanium, vanadium, and zirconium. Using tannin, hydrated tungsten(VI) oxide was ignited at 630° to 835° C. When both tannin and cinchonine were used, ignition was made at 630° to 830°. Duval (*30*) investigated in detail the behavior of filter paper during the burning process. The paper lost water but retained its form as carbon at 410°, becoming white at 675° C. Tschirch (*134*) discussed recent developments in the technique of handling precipitates, especially vacuum drying and treatment with acetone. Matheson (*85*) recorded various improvements in the technique of weighing. For the calibration of weights, Herbo (*56*) rejected the method of indirect weighing and suggested an improved procedure.

Analytical Methods for Specific Materials. Willey and Caine (*145*) recorded short, accurate procedures for the analysis of foundry sand. These authors aimed to promote analytical research to assist in solving foundry sand problems. Mohler and Sedusky (*92*) published a review of various methods used in analysis of plating baths. In the analysis of iron ores, Baron (*4*) regarded as of doubtful value the determination of "loss on ignition," by loss of weight, and preferred to determine carbon dioxide and water directly.

Touhey and Redmond (*132*) discussed the determination of tungsten, titanium, columbium, tantalum, iron, cobalt, and nickel in cemented carbide compositions. Cinchonine precipitated tungstic oxide together with titanium, columbium, and tantalum. The impure precipitate was fused and then treated with cupferron to obtain the weight of the above impurities. Deducting this weight they obtained the weight of tungstic oxide with any molybdenum present. Bowden (*13*), criticizing this procedure, stated that molybdenum is partially precipitated by cupferron and upon ignition above 525° is partially volatilized in the presence of tungsten, etc. This author also called attention to the Touhey and Redmond method of collectively precipitating titanium, columbium, tantalum, and iron in the presence of chromium and vanadium. According to Bowden, the potassium carbonate fusion used to separate these constituents would result in dissolution of a large proportion of tantalum and columbium oxides. Touhey and Redmond (*133*) acknowledged this error and introduced some alterations, but they included no reply to the criticism concerning the distribution of molybdenum. Smith (*124*) recorded detailed procedures for determining various constituents in magnesia. The methods were largely conventional but some variations were introduced. Krossin (*77*) published procedures for determining lead sulfate, lead sulfide, and lead oxide in the presence of one another. Directions were given for determining lead oxide in roast material and flue dust. Piquott (*109*) reviewed analytical techniques used in ferrous metallurgy.

General Gravimetric Reagents. Portnov (*110*) dealt extensively with the application of aromatic-arsenic compounds in chemical analysis. The dissociation constants of six compounds were given together with the insoluble salts produced by various cations. The author described two specific procedures: in one, $p\text{-AcNHC}_6\text{H}_4\text{AsO}_3\text{H}_2$ was used to precipitate cobalt in the presence of nickel; in the second, $p\text{-HOC}_6\text{H}_4\text{AsO}_3\text{H}_2$ was used to precipitate lead in the presence of alkaline earth elements. Jean (*65*) discussed in considerable detail the effect of acidity on the composition of insoluble salts formed by various metallic cations with heteropoly acids and amino bases. Conditions for analytical applications were favorable when the acidity of the precipitating medium was greater than that at which hydrolysis occurred. Cattelain (*23*) reviewed the use of organic reagents in inorganic analysis.

LIGHT ALLOY ELEMENTS

Lithium, Beryllium, and Magnesium. Boy (*14*) discussed the determination of lithium in phosphate and silicate minerals. Aluminum was removed as the phosphate. With silicates, phosphoric acid was added before addition of lead acetate. Lithium was determined as fluoride, and if magnesium was present separation from the mixed fluorides was effected by ammonium hydrogen phosphate.

For separating beryllium from aluminum, Otsuka (*100*) recommended the sodium hydrogen carbonate and the oxine methods.

Matsuura (*86*) determined magnesium in metallic aluminum by precipitation from an alkaline cyanide solution, thus avoiding interference from copper, nickel, and iron. Gaspar y Arnal and Rojo (*44*) separated magnesium from the alkaline earths and lead by precipitation as sulfites in ethanol-water medium. Ivanova and Chugunova (*63*) determined magnesium oxide in magnesium metal powder by selectively dissolving the former in a solution of chromium(VI) oxide.

Aluminum. Analytical methods for aluminum alloys (*26*) were published by the Chicago Aluminum Research Institute. Silverman (*121*) determined aluminum by 8-hydroxyquinoline (8-quinolinol). The hexamethylenetetramine precipitate was treated to remove chromium as volatile chromium dioxydichloride, followed by selective removal of iron and molybdenum by 8-hydroxyquinoline in the presence of citric and acetic acids. Lacroix (*79*) studied as a function of pH the solubilities in water and chloroform of the oxinates of aluminum, gallium, and indium. Wilson (*146*) used benzoate precipitation to determine aluminum in the presence of iron. Thioglycolic acid as reducing agent formed a soluble complex with divalent iron. Chromium, vanadium, and titanium interfered.

Gentry (*48*) published an analytical scheme for permanent magnet alloy. Following treatment with potassium cyanide in ammoniacal tartrate solution, aluminum was precipitated by oxine aided by sulfonated lorol. Smith and Cagle (*123*) determined aluminum in iron ore by precipitation with 2,2′-bipyridine which simultaneously formed a soluble complex with divalent iron. Edwards (*33*) determined aluminum in aluminum bronze by precipitation with 8-hydroxyquinoline following separation by treating the sulfuric acid solution of alloy with tartaric acid, iron (III) chloride, ammonium chloride, ammonium hydroxide, potassium cyanide, and sodium sulfite. Böttger (*12*) preferred hexamine to ammonia water for precipitation of aluminum and iron.

Ivanova and Chugunova (*63*) determined aluminum oxide in aluminum powder by selectively dissolving the oxide in chromium(VI) oxide and nitric acid. Aluminum was determined in the filtrate as hydroxide. Kimura, Kuroda, and Urakawa (*69*) determined aluminum oxide in aluminum by isolation with aqueous solution containing tin(IV) chloride, ammonium chloride, and oxalic acid.

NATURALLY RADIOACTIVE ELEMENTS

Thorium, Uranium, and Plutonium. Moeller and Schweitzer (*91*) determined thorium in monazite and concentrates by precipitation with a measured excess of sodium pyrophosphate containing a known activity of radioactive phosphorus and determining radioactivity of the excess.

Yu (*150*) described a modification of Scott's method for the analysis of uranium ore containing high content of columbium and tantalum. Duval (*32*) used the thermal balance to study optimum ignition temperatures of precipitates of uranium salts. A table of suitable temperatures for drying or calcination of precipitates was included. This author preferred precipitation of uranium by oxine or oxalic acid.

Harvey, Heal, Maddock, and Rowley (*54*) recorded various reactions of plutonium. Hydrogen peroxide precipitated tetravalent plutonium in the pH range 3 to 4.5. The composition of the precipitate was not known. Hexavalent plutonium was quantitatively precipitated by 8-hydroxyquinoline in the pH range of 3.5 to 9. Other precipitants were recorded.

ALKALI AND ALKALINE EARTH ELEMENTS

Sodium and Potassium. Klement and Dmytruk (*71*) discussed the removal of phosphate ion in preparation for sodium determinations, and recommended adsorption of phosphate by Wolfatite M. The resin required special treatment. Lund (*80*) investigated methods for the determination of sodium and potassium in silicate minerals and rocks. For low sodium contents the direct determination of sodium as triple acetate was considered superior to the indirect method of J. L. Smith, in which the sodium content is obtained by difference.

Belcher (*8*) published reviews dealing with inorganic and organic reagents for potassium.

Calcium, Strontium, and Barium. Peltier and Duval (*101*) made a critical study of about a dozen gravimetric methods for calcium in the presence of magnesium. These authors recommended the tungstate method because of its ease of execution and speed. This finding is contrary to that of Ievins and Grinsteins (*61*), who reported various difficulties with the tungstate method and stated that, contrary to the literature, this method could not satisfactorily separate calcium from magnesium. Shvedov (*120*) used radioactive indicators to determine the efficiency of the oxalate method for separation of calcium from magnesium. It was found that coprecipitation of magnesium increased with rise of temperature from 20° to 100° C. and with increasing magnesium content. Other factors such as speed of neutralizing, etc., also affected the results. It was concluded that, although coprecipitation of magnesium could be reduced to a minimum, it was never entirely prevented.

Kallmann (*67*) used hydrogen chloride–butanol solutions to separate calcium from barium and strontium. Calcium was determined as oxalate. Strontium and barium were weighed as chlorides and subsequently separated by butanol in concentrated hydrochloric acid. Correction factors were needed. Osborn (*97*) reported on the rapid separation and determination of calcium, barium, and strontium in rocks and minerals. Gaspar y Arnal and Rojo (*45*) separated calcium from barium, strontium, and lead by treatment of the sulfites in dilute ethanol with nitric acid. At a nitric acid strength of 1.43 *N*, calcium sulfite was selectively dissolved. Yatagawa and Tei (*148*) found that the determination of calcium in brass usually resulted in contamination of calcium oxalate by copper. This error was avoided when the solution, after electrolysis, was evaporated and the residue oxidized with bromine. Asari and Watanabe (*2*) recommended nitric acid of density 1.445 for the separation of the nitrates of strontium and calcium by Noll's method. When considerable calcium was present three to four reprecipitations were necessary.

Segnit (*117*) discussed the behavior of barium in silicate analysis. Complete separation of barium and calcium as oxalates was not effected in the presence of more than 4 to 5% of barium oxide. Directions were given for the recovery of barium in the presence of calcium and of magnesium. Kolarow (*72*) stated that with an experienced analyst, ignition of barium sulfate in the presence of filter paper did not result in the formation of appreciable barium sulfide. Gaspar y Arnal and Poggio Mesorana (*39*) precipitated barium, calcium, and strontium as sulfite in ethanol-water medium. Gaspar y Arnal and Santos (*47*) determined barium or thiosulfate by precipitation as barium thiosulfate monohydrate from an ethanol medium. Titration with iodine could be used as an alternative to weighing the monohydrate. Strontium interfered but calcium did not.

STEEL-FORMING ELEMENTS

Zirconium. Charlot (*25*) proposed the use of phosphoric acid in 2 to 3 *N* hydrochloric acid for the characterization of zirconium. Arsenic acid effected a selected separation of zirconium and titanium. Various organoarsenic acids were also suggested for zirconium and associated elements. Wada and Ishii (*142*) used hydrofluoric acid to determine zirconium in the elementary state in commercial samples of zirconium. This method gave results favorably comparable to those obtained by the method of weight increment upon oxidation and avoided certain difficulties. Hahn (*53*) described the precipitation and determination of zirconium by the product of hydrolysis of metaphosphoric acid and of organic phosphates. Willard and Hahn (*144*) preferred ethyl orthophosphate to diammonium hydrogen phosphate for the precipitation of zirconium. The precipitate of $ZrO[H(CH_3)PO_4]_2$ and of $ZrO(H_2PO_4)_2$ was much denser and much more crystalline. The solutions were adjusted to 3.6 *N* and ignition was continued to the pyrophosphate. Precipitations with metaphosphoric acid were preferred to the direct addition of a soluble orthophosphate. Compared to the cupferron method, determinations were more rapid and required fewer separations and manipulations.

Columbium and Tantalum. Alimarin (*1*) determined columbium and tantalum in an oxalic acid solution by precipitation with a reagent prepared from pyrogallol and urotropine. The complex was ignited at 900° to 1000° C. Slavin and Mendonça Pinto (*122*) used a modification of Schoeller's tannin method for the analysis of tantalite-columbite concentrate. The time required for an analysis was about 4 to 5 days, compared to about 15 days for the original Schoeller procedure. Rogers (*114*) discussed the principles involved in the determination of columbium, tantalum, and tungsten in steel.

Jaboulay (*64*) determined tantalum and columbium in ferrotantalum, ferrocolumbium, and steels. The alloy was fused with potassium pyrosulfate, and an extract made with sulfuric acid and sodium sulfite. The mixed pentoxides were treated with ammonium polysulfide, and after filtering and washing, the residue was calcined and the silica volatilized as usual. The separation was repeated and the pentoxides collected from the filtrate were added to the main residue. The combined precipitates were fused with potassium hydroxide and the tantalum was separated by treatment of the aqueous solution with 12% hydrogen peroxide and sulfuric acid. After one reprecipitation, columbium was removed from the filtrates by sodium sulfite and sulfuric acid. Barber (*3*) published a procedure for determining columbium and tantalum in basic Bessemer slag. The procedure was similar to Jaboulay's except that the purified residue of columbium and tantalum pentoxides was fused with potassium carbonate and the earth acids were precipitated by tannin. After final fusion with potassium pyrosulfate and extraction with water and sulfuric acid the mixture of oxides was weighed.

Maurer (*87*) published a procedure for the determination of columbium carbide in stainless steel. The sample was digested with sulfuric acid to isolate columbium carbide, which subsequently was dissolved with perchloric and nitric acids. Columbium was finally precipitated in sulfur dioxide–hydrochloric acid medium. Société Générale Métallurgique de Hoboken (*126*) recorded methods for the separation of columbium and tantalum. Columbium pentoxide was selectively reduced by hydrogen or selectively "nitrided." The nitride was treated with a halogen to form columbium halide, which was separated from tantalum by distillation and condensation.

Molybdenum. Buscaróns Ubeda and Loriente Gonzales (*20*) stated that *o*-bianisidine could be used for the gravimetric determination of molybdenum. Feigl and Raake (*34*) discussed the "masking" effect of hydrogen peroxide in precipitations of molybdate, tungstate, and vanadate. All known precipitating reactions of molybdate and tungstate could be prevented, although lead molybdate, once formed, retained its insolubility. Blanco (*11*) reviewed 22 methods for the determination of molybdenum, seven of which were gravimetric.

Tungsten. Buscaróns Ubeda, Herrera, and Loriente Gonzales (*19*) recorded three procedures which eliminated interference by carbon dioxide in the determination of tungsten by precipitation as barium tungstate. Fomin, Shalyagin, and Starostina (*37*) stated that tungsten is precipitated quantitatively by prontosil in the presence of nitric acid or ammonia water. Philipp (*105*) used an acetic acid solution of totaquinine. Furey and Cunningham (*38*) published improvements in established methods for the determination of various constituents in tungsten carbides. Brintzinger, Rausch, and Backhausen (*16*) reported a procedure for determining tungsten in ores and slags. The ores were corroded by treatment with sodium sulfate at 750° to 850° C. in a current of hydrogen or illuminating gas. After removal of silica and sulfides of divalent iron and manganese, tungstic acid was precipitated by nitric acid. Mendonça Pinto (*89*) compared the cinchonine, tannin-antipyrine, and cinchonine-tannin-antipyrine methods for the determination of tungsten in samples of Brazilian scheelite. The three methods gave practically the same results.

Vanadium, Chromium, and Manganese. Gomez Ruimonte (*50*) discussed the determination of vanadium in steel containing more than 5% chromium. Vanadate was precipitated by lead acetate in an ammonium acetate medium. The author recorded the composition of the precipitate as $2Pb_2V_2O_7.PbO$ ($5PbO.2V_2O_5$). Burriel and Barcia Goyanes (*18*) recorded the composition of lead vanadates obtained at various acidities—e.g., pH 7, $3PbO.V_2O_5$; pH 9, $5PbO.2V_2O_5$; pH 10, $2PbO.V_2O_5$.

Dupuis and Duval (*28*) reported suitable ignition temperatures for heating precipitates of chromium hydroxide, phosphate, and oxinate and of the chromates of silver, mercury, barium, and lead.

Hein and Arvay (*55*) investigated the use of $Bi_3O_5H_2MnO_4$ for the gravimetric determination of manganese. The effects due to variations in acid strength, extended heating, excess of $BiOClO_4$, etc., were studied.

Nickel. Gómez Ruimonte (*51*) dealt with the interference of cobalt in the determination of nickel in special steels high in cobalt and low in nickel. Silica and tungsten were removed prior to precipitation of nickel dimethylglyoxime in an ammoniacal tartrate medium. The precipitate was treated to remove iron, and the nickel was reprecipitated in the presence of hydrogen peroxide. Voter, Banks, and Diehl (*140*) recommended 1,2-cyclohexanedione dioxime as a reagent for nickel. Although the reagent was water-soluble, some of it was coprecipitated, causing a positive error. No satisfactory method of separating iron was found. Johnson and Simmons (*66*), who previously recorded this application of nioxime, found that the precipitate did not crystallize well and that the results were too high for gravimetric work. Peltier, Duval, and Duval (*104*) tested new analytical reagents and reactions proposed during the period 1937 to 1947 in preparation for the fourth report of the Committee of the Union Internationale de Chimie. They recommended replacing dimethylglyoxime by the dioxime of 1,2-cyclohexanedione for the precipitation of nickel, because the latter reagent had been found to react at higher acidity and to be more soluble in water. These data agree with the original work of Voter, Banks, and Diehl but there is significant disagreement as to the interference of iron. The latter authors stated that "a method for the satisfactory quantitative separation of nickel from iron was not found. The possibility of using tartrate and citrate was exhaustively investigated." Peltier *et al.* stated that interference from iron could be prevented by tartrate and that cobalt could be separated in ammoniacal ammonium chloride.

Fisher and Simonsen (*35*) used the electron microscope to study variations in crystal form with conditions of precipitation. Precipitates of dimethylglyoxime with nickel and with bismuth were used, and the authors concluded that the appearance of the crystal should be used with caution for qualitative identification.

NONFERROUS METAL ELEMENTS

Copper and Zinc. Bertiaux (*9*) selected the best known methods for the analysis of copper and copper alloys.

Yatagawa (*147*) determined zinc in brass by treating the sulfuric acid solution with aluminum. From the filtrate zinc sulfide was precipitated and determined finally by precipitation with hydroxyquinoline.

Gallium and Thallium. Charlot (*25*) separated gallium from aluminum by chloroform extraction at pH 2.0 of the gallium compound of 8-hydroxyquinoline. Lacroix (*78*) reported on some of the properties of the oxinates of aluminum, gallium, and indium. One part of gallium could be separated from 10^4 parts of aluminum by chloroform extraction of the oxinate at pH 2.0.

Raghava Rao (*112*) precipitated univalent thallium with hydrogen iodate in ethanol medium. The solubility of the precipitate was appreciable. With the aid of the Chevenard balance, Peltier and Duval (*102*) determined the temperature limits for drying or igniting compounds of thallium used for its quantitative determination. Thallium(III) oxides prepared chemically or electrolytically showed evidence of transitory formation of $3Tl_2O_3.Tl_2O$. Smith (*125*) used tetraphenylarsonium chloride to determine trivalent thallium. The white precipitate of $(C_6H_5)_4AsTlCl_4$ was dried at 110°. Interfering ions were: MnO_4^-, IO_4^-, ClO_4^-, ReO_4^-, CNS^-, NO_3^-, I^-, Br^-, and complex halides. The method was considered better than methods based on determination as thallous chromate.

Mercury and Lead. Böttger (*12*) recorded that a precipitate of mercury sulfide made in the presence of hydrochloric acid contained some $Hg_3S_2Cl_2$. A method of correction was given.

Burriel and Barcia Goyanes (*18*) studied the variations in composition of the vanadates of silver and of lead as a function of acidity. The compositions were: at pH 7, $3PbO.V_2O_5$ and $3Ag_2O.V_2O_5$; at pH 9, $5PbO.2V_2O_5$ and $2Ag_2O.V_2O_5$; at pH 10, $2PbO.V_2O_5$ and $Ag_2O.V_2O_5$. Mayr (*88*) determined lead as the salt of gallic acid in a solution buffered with sodium acetate. The precipitate was weighed directly and was appreciably soluble in boiling water. In the presence of bismuth, lead was determined after removing bismuth from a nitric acid medium as $C_6H_2(OH)_3CoOBi(OH)_2$. Murgulescu and Dobrescu (*93*) precipitated lead as basic salicylate. The dried organic complex was converted to lead sulfate and the results were found to be excellent. Young, Golledge, and Talbot (*149*) devised a method for the determination of lead oxide in ores, etc., which avoided errors due to lead phosphate and lead vanadate counting as sulfides in the separation of the latter from oxidized forms of lead. Lead sulfate, carbonate, and oxide were selectively removed with ammonium acetate. The residue was treated with perchloric acid which isolated lead sulfide. Krossin (*77*) recorded a procedure for the determination of lead sulfate, lead sulfide, and lead oxide in the presence of one another. Total lead was determined after fuming with mixed acids. To determine lead monoxide and lead sulfate, the sample was treated with ammonium acetate. Lead monoxide in roast material was extracted with ricinoleic acid and ethanol.

Gaspar y Arnal, Poggio Mesorana, and Rodriguez Calleja (*42*) determined lead by precipitation as lead sulfite in 50% ethanol and subsequently by converting to sulfate. Brut (*17*) stated that ethanol was necessary for the separation of lead as sulfate. However, the presence of sulfuric acid in the alcohol solution increased the solubility appreciably. Hubicki and Rys' (*60*) discarded tartaric acid in the determination of lead as phosphate. Diammonium hydrogen phosphate in an ammoniacal medium was recommended. The precipitate was dried at 130° C.

Tin and Germanium. Jean (*65*) precipitated tin with tungstosilicic acid in the presence of hexamethylenetetramine in the pH range 1 to 3. The precipitation was followed by a nephelometric determination by cupferron. The method was applicable to steels and was faster and more accurate than older methods.

Holness (*59*) precipitated germanium by tannin in oxalate solution at 0.07 *N* acidity. Clean separations were effected in one precipitation from vanadium, iron, zirconium, thorium, and aluminum. Tin and tantalum precipitations took place at a higher acidity and titanium at a lower acidity. The method was superior to precipitation in acid sulfate solution in that titanium could be separated and the precipitate was more tractable.

Arsenic and Bismuth. Kolthoff and Carr (*73*) recorded a procedure for the quantitative collection of traces of arsenic by coprecipitation with ammonium magnesium phosphate.

Gaspar y Arnal, Poggio Mesorana, and Rodriguez Calleja (*41*) separated bismuth and copper by a double precipitation of bismuth with nitric acid and sodium sulfite in 67.2% ethanol. The precipitate could be converted to bismuth sulfate by heating to 340° to 380° C., or by stronger heating, bismuth oxide could be formed. Jean (*65*) precipitated bismuth as a complex with thiourea and tungstophosphoric or tungstosilicic acids. The complex was dissolved and bismuth determined colorimetrically. The method was applied to certain metallurgical products. Bertiaux and Théry (*10*) stated that in the determination of bismuth in industrial lead the preliminary removal of lead as sulfate resulted in loss of bismuth. They recommended the separation of bismuth by potassium bromate in a slightly alkaline medium. A little antimony or tin or small proportions of arsenic, copper, iron, cadmium, and zinc did not interfere.

RARE EARTHS AND RELATED ELEMENTS

Scandium. Beck (*7*) reported that phytin could be used to precipitate scandium, producing a salt whose insolubility was comparable to that of barium sulfate. Titanium, zirconium, hafnium, and thorium interfered. Ostroumov (*99*) recommended a buffered solution of pyridine for precipitation of scandium. Separation from rare earth elements was effected by double precipitation at pH 4.9. Triple precipitations were required to separate scandium from gadolinium, holmium, and ytterbium.

NOBLE METAL ELEMENTS

Silver. Peltier and Duval (*103*) discussed a completely automatic method of analysis of some alloys of silver and copper. The alloy was dissolved in nitric acid and evaporated to nitrates of copper and silver, which were then heated on the thermobalance. From the magnitude of the weight losses in various temperature ranges the composition of the alloy could be determined. The error was of the order of 0.3%.

Platinum Group. Voter, Banks, and Diehl (*141*) used 1,2-cyclohexanedione dioxime (nioxime) for the precipitation of palladium. Vanossi (*138*) recorded minor improvements in a previously published method for the separation and identification of osmium, ruthenium, and germanium. Pshenitsyn (*111*) discussed the use of sparingly soluble sulfides in the separation and estimation of platinum and palladium. From a mixture of the sulfides of copper, palladium, rhodium, and platinum, ferric chloride dissolved the copper(I) sulfide completely and platinum sulfides only slightly. Ubaldini (*136*) used 2-mercaptobenzothiazole for the precipitation of platinum, palladium, and rhodium. The platinum complex was precipitated in acid medium. In alkaline solution, sodium hexachloroplatinate, in contrast with hydrogen hexachloroplatinate, did not produce a precipitate. In alkaline solution, rhodium produced insoluble $Rh(C_6H_4NSCS)_2$ while the palladium complex was precipitated in either alkaline or acetic acid media. Based on these data, Ubaldini and Nebbia (*137*) developed a method for the separation of platinum from rhodium and palladium in potassium hydroxide solution. In the presence of potassium salts, mercaptobenzothiazole precipitated rhodium and palladium. With sodium salts the precipitate was colloidal and occluded part of the platinum.

Thiers, Graydon, and Beamish (*129*) investigated by radioactive means the distribution and losses of ruthenium during fire assays. Losses to the slag and cupel were found, but there was no loss by volatilization. A new lead parting method was developed. The "insoluble" could be dissolved readily by treating the filtered residue and paper with chlorine in sodium hydroxide medium. Ruthenium was determined by "thionalide."

NONMETAL ELEMENTS

Hydrogen, Carbon, and Silicon. Sauerwald (*116*) determined hydrogen in magnesium metal by chlorination to form hydrogen chloride gas, which subsequently was oxidized with oxygen gas in the presence of cupric oxide. The water vapor was absorbed by magnesium chloride and phosphorus pentoxide.

Naughton and Uhlig (*95*) investigated the cause of the discrepancy in results for carbon in steel obtained by the standard combustion method and by the low-pressure combustion method. They concluded that the latter method met present requirements for the analysis of compounds containing little carbon. Kovtun (*76*) determined carbon in iron alloys at lowered temperatures by addition of the flux PbO(CuO)Pb. With low carbon-iron alloys the iron was largely removed by selective dissolution with K_2CuCl_4. The author also recommended the multiple combustion method for determining carbon in furnace dust and slags.

Shinkai (*118*) determined silica by precipitation with aluminum hydroxide at a mole ratio of 1 to 15. Silica was determined by difference after removal by hydrofluoric and sulfuric acids. Cavallaro and Tani (*24*) found that the silicomolybdates of trimethylamine and triethylamine were more easily filtered and washed than was the silicomolybdate of pyridine. The use of the former would facilitate determination of silicon in steels. The compositions of silicomolybdates and silicotungstates were discussed. Stross (*127*) recorded an improved gravimetric determination of silicon in aluminum alloys that was developed in Germany during the war. The sample was heated in sodium hydroxide solution in a nickel beaker, followed by treatment with hydrochloric or sulfuric acid. Copper, etc., were dissolved with hydrogen peroxide, and gelatin was added after initial flocculation of silicic acid. Phillips and Herman (*106*) determined small amounts of silicon in magnesium alloys by treatment with brominated nitric acid followed by addition of a sulfuric acid solution of hydrogen peroxide. Gelatin and pulped accelerators were used to aid filtration.

Brabson, Mattraw, Maxwell, Darrow, and Needham (*15*) developed a gravimetric procedure for the determination of silica in compounds such as fluosilicate, cryolite, and fluorspar. The silica was weighed as the oxine salt of silicomolybdic acid. Fluorine was "complexed" with boric acid. The method was suitable for the analysis of complex fluorides in which silica was a major constituent. Phosphates required a correction. Tetravalent germanium, pentavalent vanadium, and arsenic interfered. The determination of silicon and manganese in iron and steel was discussed in a report of the Methods of Analysis Committee, Metallurgy Division, British Iron and Steel Research Association (*90*).

Gurvits and Podgaits (*52*) recorded a procedure for the determination of quartz in the presence of silicates, which involved an extraction with hydrochloric acid, ignition of residue, treatment with hexafluosilicic acid, and weighing, followed by addition of sulfuric and hydrofluoric acids and a second weighing. The difference in weights was assumed to be the weight of quartz.

Phosphorus. Kesans (*68*) precipitated phosphoric acid as bismuth phosphate. Separation from various divalent cations was effected satisfactorily. Holder (*58*) determined phosphoric acid in the presence of vanadic acid by reduction to tetravalent vanadium by sulfur dioxide followed by precipitation of phosphate with magnesia mixture in a tartaric acid–ammonium chloride medium. DuBox (*27*) recorded a procedure for the rapid determination of phosphate in steel by acid-molybdate solution. An explanation was given for the increased rate of precipitation. Cale (*22*) discussed the determination of sodium pyrophosphate and triphosphate in commercial tripolyphosphate.

Oxygen and Sulfur. Pigott (*107*) discussed Gray and Sander's method of determining total oxygen in ferrous metals. The sample was heated at 1150° in contact with aluminum metal in a graphite boat, followed by determination of aluminum oxide. A single determination required several days, but the apparatus was less costly and complicated than vacuum fusion apparatus.

Kitahara (*70*) determined sulfur in "pure iron" by treating the chloride solution of the sample with ethyl ether saturated with hydrogen chloride to remove iron (III) chloride, reducing the remaining iron with zinc, and finally precipitating as barium sulfate. Isakov and Shipunova (*62*) used picric acid prior to precipitation of barium sulfate.

Mahr and Krauss (*84*) published a new method for determining sulfate. $Co(NH_3)_6BrSO_4$ was precipitated from neutral or slightly acid solutions, dried at 80° C., and weighed. As alternatives, the precipitate could be dissolved in water and the bromine content determined volumetrically, or the color of the aqueous solution could be used to measure the cobalt content.

Flint (*36*) dealt with the determination of small concentrations of sulfur trioxide in the presence of larger concentrations of sulfur dioxide. The two gases were absorbed by a mixture of water and isopropyl alcohol. The sulfur trioxide was determined as barium sulfate and the sulfur dioxide was distilled with a stream of nitrogen and absorbed in hydrogen peroxide.

Tkachenko and Khripach (*130*) described in detail a painstaking procedure for the determination of "free silicon dioxide" in ores and associated rocks.

Gaspar y Arnal and Poggio Mesorana (*40*) discussed the gravimetric determination of sulfites by precipitation of barium salts in ethanol-water medium, followed by conversion to barium sulfate. Sulfur trioxide in soluble sulfites was determined gravimetrically by precipitating by lead nitrate in ethanol-water medium followed by conversion to lead sulfate.

Selenium and Tellurium. Tananaev and Murasheva (*128*) determined selenium in steel by dissolving the residue from a sulfuric acid treatment in nitric acid and converting to chlorides. The selenium was precipitated with sulfur dioxide. Ghosh (*49*) determined selenium and tellurium in refined copper by dissolving the sample and iron filings in nitric acid, adding ammonia water, and from a hydrochloric acid solution of the mixed hydroxides removing selenium and tellurium by stannous chloride.

Fluorine. Wiechert and Burandt (*143*), dealing with the determination of fluorine, found that the precipitate of calcium fluoride was rendered more "filterable" if made in the presence of gelatin. Mixed precipitates of calcium sulfate and calcium fluoride were treated with orthoboric and perchloric acids to volatilize boron trifluoride. Tsubaki (*135*) published an improved method for the analysis of the mineral fluorite. The sample was fused with potassium sodium carbonate and titanium dioxide, silica was removed, and calcium fluoride was precipitated by a known amount of calcium acetate. The fluorine content was obtained by determining the excess of calcium. Rinck (*113*) published a review dealing with the separation and determination of fluorine.

Chlorine and Bromine. Pil'nik (*108*) determined chlorides by titration with mercurous nitrate followed by quantitative recovery of mercury from the precipitate.

Sampey, King, and Blitch (*115*) published an approximate method for the determination of bromine. The bromine compound was dissolved in acetone saturated with sodium iodide. The sodium bromide was determined with an accuracy of 97 to 99%.

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Merck Analytical Fellowship

Applications are being received for the Merck graduate fellowship in analytical chemistry, financed by Merck & Co., Inc., Rahway, N. J., and administered by the AMERICAN CHEMICAL SOCIETY. The annual stipend is $2500. The place of study must be an institution whose undergraduate course of instruction in chemistry is approved by the Society, or in Canada, by the Chemical Institute of Canada.

A fellowship will be awarded to the applicant believed capable of contributing most to the advancement of the theory and practice of analytical chemistry during the fellowship and in the future. It will be contingent upon the successful candidate's obtaining acceptance from the institution and professor selected for the study program proposed.

Application blanks may be obtained from the AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N.W., Washington 6, D. C. Deadline date for receipt of application, letters of recommendation, and transcripts of credits is February 15, 1950.

ORGANIC GRAVIMETRIC ANALYSIS

JOHN F. FLAGG
Knolls Atomic Power Laboratory, General Electric Company, Schenectady, N. Y.

THE subject of organic elementary analysis, with emphasis on the determination of carbon, hydrogen, nitrogen, sulfur, halogen, and oxygen has been reviewed briefly but critically (*7*). In the same article there appears a description of the adaptation of the carbon and hydrogen method of Tunnicliff *et al.* (*17*) and Wagman (*19*) for 50- to 75-mg. samples.

The material is burned in air rather than pure oxygen; the advantages claimed are greater degree of control and freedom from the risk of explosion. Air for the combustion is passed over copper oxide at 850° C., through Ascarite and Dehydrite, and thence to the combustion train. The sample, contained in a quartz combustion tube, is distilled at such a rate as to require 20 to 30 minutes for burning; a heater kept at 700° C. between the sample and the air-purifying section of the train prevents back-distillation. The tube is packed with copper oxide (850° C.), silver vanadate (400° C.) for removing sulfur and halogen, and lead peroxide (190° C.) if nitrogen oxides are present. The absorption train consists of a Dehydrite-filled U-tube, and an Ascarite-filled vertical absorption tube. It is claimed that no substance has been encountered that cannot be burned, and data on hydrocarbons, substituted aromatic acids, and some miscellaneous compounds show an attainable standard deviation of ±0.2% on carbon and ±0.1% on hydrogen.

The empty tube combustion method continues to attract study, and results of a critical examination of the method have been published (*10*). Satisfactory results were obtained using the method as originally described, but on certain research compounds, particularly volatile liquids and hydrocarbons, incomplete combustion occurred. Low carbon and slightly high hydrogen values were traced to a fine carbon fog which passed through the combustion tube and was retained in the water absorbent. Lengthening the combustion tube to 30 cm. from the recommended 25 cm., and insertion of a plug of quartz wool to trap carbon fog, make the method applicable to all types of compounds, and as efficient as the standard Pregl method. Among the 25 compounds for which data are given are picric acid, dimethylglyoxime, *p*-diacetoazoxybenzene, DDT, tetrabromodiphenylamine, sulfonal, and *N*-acetyl sulfanilamide.

The effectiveness of an external absorbent for nitrogen oxides is confirmed, although the rapid deterioration of the permanganate–sulfuric acid solution used makes renewal necessary after every two or three combustions, and points to the need for further investigation of such absorbents.

The empty tube method, as investigated under other conditions (*5*), was considered to be too narrow in range of application to meet the requirements of general routine analysis. Accordingly, the method has been modified, and the sample is burned in a twelve-turn silica helix 24 cm. long, 4 cm. in external diameter, and heated to 800° C. through which oxygen is passed at the rate of 40 ml. per minute. Oxides of nitrogen are removed by passage through lead peroxide heated to 180° C., halogen and sulfur on granulated silver at 600° C. Results are given for 35 compounds, compared with those obtained in some cases by the Pregl method, and there appears to be a slight tendency toward high results for hydrogen as found by the new method. It is emphasized that the method is regarded as being on trial. Speed is an advantage claimed for the method, with 30 to 40 minutes needed to determine carbon and hydrogen.

A simplified method for routine semimicrodetermination of carbon and hydrogen has been reported (*12*) for use on liquid samples, in which the sample is slowly volatilized and the vapors are burned in a rapid (300 to 350 ml. per minute) oxygen stream at the tip of the containing ampoule. The accuracy of the method is of the order of 0.06% for carbon and 0.08% for hydrogen, comparing favorably with other methods.

Determination of carbon and hydrogen simultaneously with the heat of combustion of an organic compound is reported (*3*). The heat of combustion is determined in a Berthelot bomb in the usual manner, and the products are passed through an absorption train to collect the carbon dioxide and water.

A high temperature gas burner has been described (*2*) for use in the thermal decomposition method of direct oxygen analysis in organic compounds. The burner may also be used in other combustion micromethods, and has the advantage of longer life than electrically heated burners at the high temperatures used (900° to 1000° C.).

The adaptation of the relatively long (33 cm.) heavy duty combustion furnace to the analysis of semimicroquantities (15 to 25 mg.) of organic materials has been described (*18*); this avoids difficulties in attempting to use the shorter (18 to 20-cm.) microfurnaces for burning semimicrosamples.

The lamp method as applied to the determination of sulfur has been studied critically (*11*) and it has been shown that low and erratic recoveries of sulfur, ranging from 19 to 63% on a 0.0117% solution of elemental sulfur in a toluene–isopropyl alcohol mixture, are obtained, and concluded that the lamp method is not quantitative for elemental sulfur, and hence not quantitative for total sulfur unless elemental sulfur is known to be absent. Complete recovery of sulfur from thiophenol, thiophene, or carbon disulfide is possible when the material is dissolved in isopropyl alcohol so as to give a nearly nonluminous flame.

Results of further studies of burner modifications have been reported (*20*), also with respect to sulfur determination. In addition, the determination of chlorine and bromine (in gasoline), normally unsatisfactory in the conventional A.S.T.M. burner, is possible in the modified burner described.

Other modified lamp designs (*9*) permit the burning of petroleum fractions in the boiling point range 250° to 500° C. The accuracy of the determination of hydrogen is ±0.02 to 0.03% absolute, and possible extensions of the method for the simultaneous determination of carbon and sulfur are suggested.

Adaptation of the Van Slyke and Folch wet combustion method for carbon to the simultaneous determination of total and radioactive carbon in biological materials has been reported (*13*). The carbon, obtained as barium carbonate following evolution of carbon dioxide by the oxidizing mixture, is determined gravimetrically with a precision of 4 to 7 parts per thousand, and its radioactivity to approximately 2%.

MISCELLANEOUS DETERMINATIONS

The use of picric acid as a precipitant for piperazine, $(CH_2)_4(NH)_2$, has been recommended (*14*). The neutralized sample is treated with a saturated solution of picric acid, whereupon piperazine picrate is precipitated. Following digestion, the precipitate is filtered, washed with saturated piperazine picrate in water, then alcohol and ether, and dried. The precipitate contains 15.81% anhydrous piperazine, or 35.66% piperazine hydrate.

The method is applicable to dimethyl piperazine, and may also be used in the presence of hexamethylenetetramine, provided that substance is first hydrolyzed with dilute sulfuric acid

The determination of mandelic acid, $C_6H_5CH(OH)COOH$, using periodic acid has been reported (*6*). Oxidation with 0.1 *N* periodic acid at 100° C. converts mandelic acid quantitatively to carbon dioxide and benzaldehyde; the latter is precipitated and determined as the phenylhydrazone following steam distillation from the reaction mixture. The results are low by a constant amount, corresponding to 5 mg. of benzaldehyde.

o- and *p*-cresols have been determined gravimetrically by the cresoxyacetic acid method (*1*).

The alkaline sample of the cresols is treated with chloroacetic acid, precipitating sodium *p*-cresoxyacetate. This salt is filtered, dissolved in hot water, and acidified with hydrochloric acid to liberate *p*-cresoxyacetic acid which precipitates upon cooling. The yield is determined by weighing, the purity by determining the melting point.

The filtrate from the *p*-cresoxyacetate precipitation is acidified and extracted with ether, from which *o*-cresoxyacetic acid is recovered. From the weight and melting point of this acid the yield of pure acid is calculated, making reference to mixed melting point curves of the two acids. An accuracy of 1.5 to 2% for the *o*- and *p*-cresols, respectively, is claimed.

Caffeine has been determined in coffee by extracting the ground sample with very dilute (<0.01%), boiling permanganate, followed by addition of copper sulfate (*16*). The filtrate and washings, made alkaline and saturated with sodium chloride, are extracted repeatedly with chloroform; the caffeine remaining after evaporation of the solvent is dried at 180° to 190° C.

Mixtures of trihydroxyglutaric, oxalic, and nitric acids resulting from the oxidation of xylose by nitric acid have been analyzed (*4*). Total acid is first determined by conductometric titration with alkali; oxalate is precipitated with calcium in slightly acid solution, and trihydroxyglutaric acid precipitated as the neutral calcium salt from cold solution at pH 7 to 8 (or preferably with heating at pH 4 to 4.5, although how this gives a sharp separation from oxalate is not clear).

A critical study of the Munson and Walker method for determining lactose, either alone or in the presence of sucrose, reveals that the tables based on the weight of cuprous oxide obtained are in error (*8*). This results from the occlusion of inorganic and organic impurities, even when pure sugars are determined, by the cuprous oxide; consequently, if the reduced copper is determined electrolytically, a lower equivalent quantity of cuprous oxide is found than when the cuprous oxide is weighed directly. The contamination increases with increasing sugar concentration, and is less marked with lactose than with lactose-sucrose mixtures; results are high by as much as 9 mg. of copper in the latter case. New tables for lactose hydrate, and for its mixtures with sucrose, are given.

A gravimetric method for the determination of choline chloride and choline citrate (*15*) is based on the precipitation of the cadmium chloride complex, $(CH_3)_3NClC_2H_4OH.CdCl_2$, from alcoholic solution. The method is of higher accuracy and precision than previous spectrophotometric or volumetric methods, as shown by analyses on solutions free from possible interferences. Where the composition of the sample is unknown, it is recommended that a nitrogen determination be made on the precipitate, as foreign nitrogen compounds appear to be precipitated. The method is recommended primarily for use on pharmaceutical grade material, and has not been investigated for biological samples.

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INORGANIC VOLUMETRIC ANALYSIS

CLEMENT J. RODDEN
U. S. Atomic Energy Commission, New Brunswick, N. J.

THE review for the year 1949 follows the pattern of the January 1949 review (*111*), with omission of most work on indicators and electrometric methods of analysis because these are covered by other authors. In general, the work during the past year has consisted chiefly of variations and adaptations of existing methods.

A considerable number of papers have appeared in the Russian literature, but unfortunately all articles are in the Russian language. Much of this work is adaptation of existing methods to specific problems.

BOOKS AND REVIEWS

Several books have appeared during the past year, among which are "Organic Reagents for Use in Volumetric Analysis" (*42*), "Aquametry" for the determination of water by Karl Fischer reagent (*89*), and one on microanalytical methods (*88*).

Of the reviews, those on the following subjects are of interest: modern practice in volumetric analysis (*43*), newer techniques (*92*), microgram procedures (*6*), sulfate by microvolumetric means (*136*), use of 8-hydroxyquinoline (*29*), and a brief discussion of the analytical work on the Manhattan District Project (*44*).

STUDIES OF METHODS

There have been a considerable number of papers in the nature of studies on existing methods, among which are titrations of cerous ion with potassium permanganate (*53*); mechanism of the bichromate-iodide reaction in acid solutions (*97*); iodometric determination of copper (*60*); separation and determination of fluorine (*110*); methods for the determination of fluorine on a macro scale (*110*), and micro scale (*143*); microdetermination of iron (*34*); determination of magnesium with 8-hydroxyquinoline (*41*); conductometric determination of mercurous iron (*48*); micromethod for potassium using cobaltinitrite (*13*); determination of peroxide with permanganate (*65*); errors in acidimetric titrations resulting from absorption by filter paper (*112*); errors in hydrolytic titration of lead nitrate (*64*); influence of acid on the oxidimetric determination of antimony (*99*); determination of carbon dioxide (*98*); studies on complex ions of aluminum, gallium, and indium (*72*); studies on the reduction of uranium compounds (*10*); and a discussion of the analysis of cadmium and cadmium plating solutions by various methods (*127*).

NEW AND NOVEL METHODS

Among the new and novel methods which have been employed are: titration of free acid in the presence of iron salts by first reducing the iron to Fe (II) in a silver reductor (*115*); precipitation titration of azides using adsorption indicators and working under an ultraviolet light (*61*); microvolumetric determination of bismuth by precipitation as iodate or chromate, followed by an iodometric determination of excess precipitant (*122*); phosphate titration of several metals which may not be very applicable because of interferences (*71*); conductometric microdetermination of carbon in aluminum after oxidizing the methane liberated to carbon dioxide, followed by adsorption in sodium hydroxide (*12*); titration of cerium ions with permanganate (*53*); cuprous ion by precipitating with thiocyanate which is dissolved in ferric sulfate, the ferrous sulfate liberated being titrated with dichromate (*17*); use of liquid amalgam and a few drops of chromous chloride to reduce iron solutions (*31*); determination of iodine iodometrically after oxidizing with chlorine water and then reacting the excess chlorine with potassium cyanide (*120*); titrating ferric chloride solutions with tartrate using calcium chloride to prevent hydrolysis (*94*); using chloramine-T to titrate ferrous iron (*1*); phototurbidimetric determination of magnesium in dolomite (*108*); formation of trivalent manganese complexes with subsequent titration with ferrous sulfate for the determination of manganese (*146*); determination of nitrate by reduction with chromous ion (*77*); using ether in the titration of sodium citrate with hydrochloric acid (*138*); and a volumetric method for the determination of silicon which appears to be rapid and to give good results (*148*).

APPARATUS

Several different kinds of apparatus have been described for use in conductometric titrations (*9*, *67*, *91*). An apparatus has been described which can be used for automatic potentiometric titration (*76*). Several pipets and burets have been described, among which are an automatic pipet and buret (*135*); pipets of high speed and high accuracy (*109*); a microburet for transferring known volumes of paste materials (*3*); and a high precision ultramicroburet using a micrometer gage dial (*51*). Among useful aids are a rotary stirring device for microtitrations (*130*); an apparatus for the rapid calibration of volumetric flasks (*15*); and an apparatus which will absorb boron when analyzing metals (*7*).

STANDARDS AND REAGENTS

Little has been done in the past year on new reagents. Ferrous propylenediamine sulfate has been recommended as being stable enough for use as a primary standard (*93*). Evidence has been submitted to show that ammonium hexanitratocerate may be used as a primary standard of oxidimetry (*128*). A historical discussion of the use of potassium dichromate as a volumetric reagent has been published (*21*). The use of silver metal in the buret used to dispense ferrous solutions is of interest (*116*). Photometric means have been used to standardize permanganate solution but appear to be of no special advantage (*2*). It has been noticed that certain salts, notably copper and molybdenum, cause air oxidation of tetravalent uranium, resulting in errors when titrating with permanganate (*10*).

Of the various reagents suggested, the following are of interest: methylene blue in the titration of molybdenum (*150*); a standard solution of dichromate in the determination of nickel (*55*); standard arsenite for the determination of chromate ion (*133*); methyl alcoholic solutions in the titration of 8-hydroxyquinoline with bromate (*100*); replacing of silver nitrate by mercuric nitrate in the determination of chlorides (*139*); the use of sodium vanadate in place of potassium dichromate for titrating iron (*107*); and the use of ruthenium salts as catalyst in the manganometric determination of tellurous acid (*73*).

DETERMINATIONS BY ELEMENTS

Acids. Free acids have been determined in the presence of iron salts after reducing the Fe(III) to Fe(II) with silver (*115*); nitric acid in mixed and waste acid by electrometric titration with ferrous ammonium sulfate (*131*); hydrochloric acid in distilled water by potentiometric titration with barium hydroxide (*33*).

Aluminum. A rapid electrometric method employing titration with sodium fluoride has been used for ore analysis (*28*). Several papers have appeared on use of 8-hydroxyquinoline in the analysis of bronze (*38*), silica brick (*80*), silicates (*144*), and other materials (*100*).

Antimony. The iodine monochloride end point is suitable for titrating antimony with potassium iodate or potassium permanganate. Ceric sulfate is not recommended (*59*). The effect of acid concentration on the oxidimetric determination of antimony using permanganate has been studied (*99*). Pentavalent antimony can be reduced completely to trivalent antimony by use of nickel powder (*63*).

Arsenic. The iodometric titration of arsenic can be made in solutions from pH 7 to 11 (*81*). Methods for the separation of tertiary arsines in mixtures have been developed (*105*).

Bismuth. A phosphate titration of bismuth has been suggested, but there are many interferences (*71*). A volumetric micromethod has been used in which the bismuth is precipitated as iodate or chromate, followed by iodometric determination of the excess chromate or iodate (*122*).

Boron. Variations of the usual titration employing mannitol are: in ferroboron (*113*), in metals and alloys (*7*), and for traces of boron (*117*).

Bromine. A method for the microdetermination of bromide ions has been described in which the bromine is converted to bromine chloride by chlorine water, potassium cyanide is added, and the bromine cyanide is then titrated in the usual iodometric manner (*120*). In table salt bromine has been determined iodometrically (*22*).

Cadmium. A discussion of methods used in analyzing cadmium cyanide plating solutions describes various methods for determining cadmium (*127*).

Carbon Dioxide and Carbon. The usual barium hydroxide adsorption after converting to carbon dioxide is followed by titration with hydrochloric acid (*98*) and oxalic acid (*118*). The carbon in aluminum is determined by conductometric titration of the carbon dioxide formed on ignition (*12*). One method employs potentiometric titration with barium hydroxide (*33*).

Carbon Monoxide. Carbon monoxide has been determined by oxidizing to carbon dioxide, absorbing in standard barium hydroxide, and titrating the excess with oxalic acid (*75*).

Calcium. Active calcium oxide in lime has been determined by boiling with sodium carbonate, followed by titration of the excess sodium carbonate and sodium hydroxide with acid (*49*). There have been several applications of the usual calcium oxalate permanganate titration to the analysis of magnesia (*129*), sinter cake (*142*), and silicates (*144*), and for the determination of small amounts of calcium (*70*).

Cerium. The direct determination, both potentiometric and amperometric, has been made of cerium ions with permanganate in the presence of pyrophosphate (*53*).

Chlorine. Determinations of chlorine in the presence of hypochlorite, chloride, and chlorate ions using arsenite have been made (*78*), as well as determinations of all the above constituents in mixtures (*85*). As an economy measure mercuric nitrate has replaced silver nitrate in chloride titrations (*129*).

Chromium. The usual persulfate oxidation followed by titration with ferrous sulfate has been applied to the semimicrodetermination of chromium in ferrous metals (*50*), steels (*54*), cast iron (*45*), and ferrochromium (*74*). For the direct determination of chromate ion, standard arsenite has been used with manganous sulfate as a catalyst and diphenylamine as an indicator (*133*).

Cobalt. The cobalt is precipitated as the arsonate after dissolving in hydrochloric acid and is determined bromometrically; nickel, however, causes some error (*102*).

Copper. A semimicro variation has been made in the usual iodometric determination (*36*). In place of the usual iodometric determination, sodium bromide has been used to convert cupric ion to bromide complex ion which can be titrated with thiosulfate (*39*). Copper has been titrated with thiosulfate in the presence of fluoride ion (*37*). A method employing dichromate, in which the copper is precipitated as cuprous cyanide followed by dissolving in ferric sulfate and subsequent titration of the ferrous sulfate with dichromate, has been reported (*17*). Traces of copper have been determined with dithizone titration (*117*), which also has been employed for copper determinations in steel (*132*).

Cyanide and Cyanate. Cyanide has been determined by the usual silver titrations (*79*), and cyanate has been determined by a rather involved reaction in which a final titration with sodium hydroxide is employed (*106*).

Fluorine. Although chlorofluoride methods have been used in ores (*101*), studies (*110, 143*) show that the Willard and Winter (*147*) method is superior. This method has been applied to the determination of fluorine in beryllium compounds (*26*) and in zirconium (*27*). A method has been suggested for the direct determination of fluorine by titrating with standard ceric nitrate solution in the presence of potassium ferricyanide (*104*).

Hydrogen Peroxide. The determination of hydrogen peroxide with permanganate has been compared to the gas measurement method (*65*). In the determination of hydrogen peroxide by iodometry, low results are sometimes obtained (*47*).

Iodine. Iodine has been determined iodometrically after oxidizing with chlorine water and binding the excess chloride with potassium cyanide (*120*).

Iron. An alkalimetric method has been described in which ferric chloride is treated with tartrate and titrated with alkali using calcium chloride to suppress the hydrolysis of the ferric chloride (*94*). Both zinc amalgam (*31*) and chromous chloride have been used to reduce ferric ion (*32*). A standard chloramine-T solution has been used to titrate ferrous iron (*1*). An extension of the work on the determination of ferrous salts in the presence of thiocyanate (*18*) has resulted in methods for the simultaneous determination of ferrous and ferric ions (*19*) and the determination of iron in minerals and alloys (*20*). The usual dichromate titration has again been the subject of several papers (*5, 23, 141*). The iodometric titration has been reinvestigated (*57*) and has been used in a microchemical method for the determination of iron (*34*) as well as for the analysis of silicates (*144*). The permanganate method has been used for the rapid determination of titanium and iron by using methylene blue for the titanium end point and *o*-phenanthroline for the iron end point (*125*). In the titration of solutions containing hydrofluoric acid with permanganate better results are obtained if phosphorus acid is added (*96*).

Lead. Small quantities of lead are precipitated as chromate and then determined iodometrically after dissolving the chromate (*69*). By precipitating with aromatic arsonates, followed by a volumetric titration, lead can be determined in the presence of calcium, barium, or strontium salts (*103*). In the iodometric titration with sodium phosphate results were shown to vary with the pH (*64*).

Magnesium. A rapid alkalimetric method has been used in carbonate ores in which the sample is dissolved in 0.5 N hydrochloric acid which is then divided and one portion is titrated with sodium hydroxide while the other portion is used for the calcium hydroxide content (*114*). The use of oxine has been studied (*41*) and used in the analysis of silicates (*144*). In a rapid method developed for aluminum alloys, magnesium is precipitated with phosphate ions followed by dissolving in acid and titrating with sodium hydroxide (*52*). A phototurbidimetric titration has been used for the analysis of dolomites by precipitating magnesium as ammonium phosphate (*108*).

Manganese. A variation of the arsenite titration after oxidizing with persulfate has been employed in the analysis of cast iron (*11, 84*), sinter cakes (*54*), iron ores (*140*), and other ores (*8*). The pyrophosphate complexes have been titrated electrometrically with ferrous sulfate (*146*).

Mercury. A study of the complexes formed during the cyanide titration has resulted in a procedure for mercurous ions using conductometric methods (*48*).

Molybdenum. In the determination of molybdenum in steel, when the amount is greater than 5%, the molybdenum is extracted as a thiocyanate, followed by reduction with zinc amalgam and titration with potassium permanganate (*149*). In the analysis of ferromolybdenum steel the molybdenum is reduced with zinc amalgam and then titrated with methylene blue; the results are said to be more reproducible than in the usual volumetric method (*150*).

Nickel. The determination of nickel by titrating with dimethylglyoxime is said to be suitable for steels containing up to 3% copper (*55*).

Nitrogen. For the determination of azides the sample is oxidized with permanganate followed by an iodometric titration (*86*). Azides may also be titrated with silver nitrate using adsorption indicators (*61*). A method for the determination of nitrate by reduction with chromous ion followed by titrating excess chromous ion with ceric sulfate is useful in the 20- to 50-microgram range (*77*).

Phosphorus. The only thing that has appeared is a variation of the alkalimetry titration for phosphorus in cast iron (*11, 126*), phosphate rock (*68*), and basic slag (*24*).

Potassium. A semimicrodetermination of potassium based on precipitation as potassium sodium cobaltinitrite, followed by oxidation with dichromate and subsequent titration of excess dichromate with ferrous sulfate, has been recommended (*13*).

Rhenium. A very interesting discussion on the conditions for the reduction of rhenium to various states should be of assistance in the determination of this element (*137*).

Selenium. A variation of a method employing extraction in chloroform followed by titration with thiosulfate (*40*) has been used in lead alloys (*14*). A method which has been stated to be simpler than the American Society for Testing Materials' method consists of precipitating the selenium with hydrogen sulfide, dissolving in nitric acid, and following with an iodometric titration in the presence of urea (*134*).

Silicon. A new and interesting volumetric method for silica which may be of considerable assistance employs precipitation of silicon as quinoline silicomolybdate followed by dissolving in standard sodium hydroxide and then titrating with hydrochloric acid. The method is rapid and the results appear satisfactory (*148*). Another alkalimetric titration follows the precipitation of silica as potassium silicofluoride (*16*).

Sulfur. A simple procedure for the determination of hydrosulfite consists of titrating with ferric chloride, using a mixture of thiocyanate and ferrocyanide as an indicator (*56*). In the titration of persulfate, ferrous sulfate has been suggested as a catalyst instead of molybdenum (*35*). For the sulfate determination there has been a review of microvolumetric methods (*136*) and an iodometric method for its determination in the presence of calcium (*95*). In a variation of the usual procedure for the determination of sulfur in sulfidic ores the roasting gases are collected in peroxide and titrated with sodium hydroxide (*119*). For the titration of thiocyanate it has been pointed out that when an iodate solution is used, the results obtained are quantitative, whereas they are not if permanganate or ceric sulfate is employed (*58*). This is in contradiction of a paper (*123*) which determines thiocyanate by permanganate oxidation in the presence of halogens.

Tellurium. Ruthenium has been suggested as a catalyst in the manganometric determination of tellurium (*73*).

Thallium. An interesting procedure, which needs some additional investigative work, consists of precipitating thallium iodide using bromophenol blue as an adsorption indicator (*83*).

Tin. In the analysis of tungsten high-speed steels (*124*), the tin is precipitated as sulfide, reduced with aluminum, and titrated with potassium iodate (*4*). This titration has also been used for the analysis of copper-based alloys (*87*).

Thorium. By modifying the iodate method of Chernikhov and Uspenskaya (*25*) workable procedures have been developed (*90*, *145*).

Titanium. For the rapid determination of titanium, titration with permanganate using methylene blue has been suggested (*125*). Variations in the usual silicate analysis methods have appeared wherein the titanium is titrated with sodium dichromate (*144*). For titanium in ferrous alloys and steels the use of zinc amalgam as a reductor has been suggested (*151*).

Vanadium. A variation in the standard method for the determination of vanadium (*62*) consists in using the dead-stop titration method for milligram quantities (*46*). The ferrous sulfate reduction for use in ferrous metals (*50*), steel (*54*), vanadium (*66*), and chrome steels (*30*) has again been published.

Water. In the determination of water the book "Aquametry" gives a literature survey as well as theory and practice (*89*). A variation of the Karl Fischer method which is stoichiometric may be of interest (*121*).

Zinc. The only addition to the literature on zinc has been a study on the iodometric determination by an empirical method (*82*).

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Volumetric Analytical Methods for Organic Compounds

WALTER T. SMITH, Jr., and RALPH L. SHRINER
State University of Iowa, Iowa City, Iowa

The following discussion concerns volumetric methods for quantitatively determining elements and functional groups of organic compounds as well as procedures for estimating the percentages of compounds in mixtures. The literature covered extends from October 1948 to October 1949 and includes items from *Chemical Abstracts* for this period from certain foreign journals of prior years.

Examination of many of the articles in foreign journals shows that they have not been able to obtain or read the American publications for many years. Many of the procedures reported have been in use here for many years. Such articles have been cited briefly but do not represent new contributions to the field of organic analysis.

ANALYSES FOR ELEMENTS

BORON

An adaptation of the method of Burke (*11*) for determining boron in organic borates has been developed for the determination of boron in borine compounds (*18*). The sample is weighed in a gelatin capsule in an atmosphere of nitrogen and is burned in a Parr oxygen bomb under a pressure of 500 pounds per square inch. A sodium carbonate solution is used to absorb the combustion gases. The acidified bomb washings are boiled with potassium permanganate to complete the oxidation, and the resulting boric acid is determined by the standard procedure of removing carbonate and titrating with sodium hydroxide in the presence of mannitol. The complete procedure requires only 3 hours, of which one hour is actual working time.

A micromethod (*1*) for determining boron in organic compounds uses a digestion of the sample with a mixture of nitric, sulfuric, and perchloric acids. After dilution, urea is added to remove nitrous oxides and the solution is carefully neutralized to methyl red. Mannitol is then added and the titration with standard alkali is carried to the phenolphthalein end point.

A detailed procedure (*83*) for determining boron in plants and soils has been described, which also uses the mannitol titration as the determining step. Copper in plants and soils is also determined volumetrically by titration with dithizone after suitable destruction of the organic compounds. The same procedure should be useful for analyzing organo-copper complexes.

HALOGENS

Polymers containing chlorine (*73*) may be decomposed by heating the sample in a tube through which oxygen is passed. The combustion products are passed over a platinum catalyst and then over barium carbonate. The latter absorbs the hydrogen chloride to give barium chloride, which is titrated with standard silver nitrate solution.

In a modification of the method of Schwenk, Papa, and Ginsberg (*85*) zinc and alkali are used to remove halogen from organic compounds (*70*). In order to effect complete removal of halogen, a catalytic amount of Raney nickel alloy is added. In this way all of the halogen can be liberated in 1 hour on a boiling water bath. An excess of silver nitrate is added and back-titrated with potassium thiocyanate. Among the compounds which have been satisfactorily analyzed by this method are *p*-chloro-*m*-

cresol, p-chloronitrobenzene, tribromophenol, bromocamphor, bromotriphenylethylene, bromosuccinimide, eosin, dibromotyrosine, diiodotyrosine, diiodothymol, and the benzoate of dibromo-2,4-androstanol-17-one-3.

In order to determine halogens in organic compounds a procedure using preliminary decomposition of the sample in a bomb with hydrazine hydrate has been devised. The halide ions are subsequently determined by the usual volumetric Volhard titration. Modifications of the various titrimetric methods using selective oxidizing agents are described for determining the halogens in the presence of each other (*47*).

Fluorine in organic compounds (*17*) is determined by decomposing the sample with sodium peroxide in a bomb and dissolving the melt in water. After boiling to decompose peroxides, the fluoride ion is determined by the lead chlorofluoride method with a Volhard finish, using a correction curve. Monofluoroacetamide is suggested as a standard reference substance.

Iodine in organic compounds (*8*) may be determined by oxidation of the sample with potassium permanganate at pH 3.2. The iodine is converted to iodate, which is then titrated iodometrically.

NITROGEN

A bromometric method (*6*) for the determination of nitrogen depends upon the oxidation of the nitrogen of organic compounds to free nitrogen by treatment with a known volume of potassium bromate solution together with potassium bromide and dilute sulfuric acid. After a little while, potassium tetraborate is added to convert the excess bromine to hypobromite. Then excess potassium iodide is added and the solution is titrated with sodium thiosulfate to determine how much potassium bromate was necessary for complete oxidation of the nitrogen in the compound.

Noyce (*64*) has described a modified Dumas nitrogen method which reduces the time required for the usual macroprocedure to about one half and gives excellent results in the hands of inexperienced operators. The method provides for a convenient carbon dioxide source, the use of a back-flush technique, and provision for cooling the combustion tube outside the furnace.

The use of dry ice in a Dewar flask fitted with a capillary, as a source of carbon dioxide in the Dumas determination of nitrogen, has been known and used in the United States for at least 25 years but it has just been discovered abroad (*84*).

Studies of the Kjeldahl method for determining nitrogen in proteins (*43*) show that only the mercury-catalyzed decompositions give results as high as the Dumas method.

Micro and semimicroprocedures for determining heterocyclic nitrogen by the modified Kjeldahl method are given (*65*) and a new type of connecting bulb (*110*) has been described for use during the distillation of the ammonia. The bulb contains two wire screens which form water films and prevent carrying over of alkali and also serve as an effective antifoaming trap.

OXYGEN

The method of Unterzaucher (*104*) for the determination of oxygen in organic compounds in which the sample is heated in pure nitrogen and the volatile products are passed over hot carbon to form carbon monoxide which is oxidized by iodine pentoxide to carbon dioxide and iodine, has been improved (*106*) by replacing the nitrogen by helium and by collecting the carbon monoxide in a flask of known volume and determining it by the palladium silicomolybdate colorimetric method described by Shepherd (*88*). Using these modifications, compounds containing from 0.01 to 0.6% oxygen can be analyzed satisfactorily.

The Unterzaucher method (*104*) has also been adapted to the determination of oxygen in petroleum compounds (*22*). By using larger samples, titration errors have been minimized for those cases where the oxygen content is low. The use of a special adapter assembly permits more efficient forward and backward nitrogen sweeping and also permits the elimination of the conventional side arm of the combustion tube. It has also been found possible to substitute an inexpensive commercially available furnace for the special furnace previously used. The presence of nitrogen and sulfur in compounds to be analyzed causes no interference. The accuracy obtained with the new apparatus is about 1% of the oxygen content. A determination may be run in about 70 minutes.

SULFUR

Elemental sulfur in hydrocarbons has been determined by a modification of the method of Oldham, Baker, and Craytor (*66*) for determining sulfur in rubber (*61*). The method is based on the equation:

$$S + Na_2SO_3 \longrightarrow Na_2S_2O_3$$

The method has been used for free sulfur in benzene, toluene, ethylbenzene, styrene, and other aromatic hydrocarbon mixtures and gives satisfactory results when the sulfur is present in the range 1 to 20%. When 100-ml. samples are used, the accuracy ranges from ±0.002% for samples containing 0.1% free sulfur to ±0.4% for samples containing 20% free sulfur. Efforts to adapt this method to the determination of 0 to 100 p.p.m. of free sulfur in styrene gave erratic results. Efforts to apply the butyl mercaptan (butanethiol) method (*103*) to samples containing 10 p.p.m. of free sulfur gave fairly uniform results, but they averaged about 172% high (*5*).

Elving and Ligett (*26*) described a method for decomposing organic halogen compounds with sodium or potassium at 400° C. in an evacuated tube. This decomposition procedure has now been adapted to organic sulfur compounds and leads to a method for determining sulfur quantitatively (*49*).

The results and possible sources of error in determining carbon, hydrogen, nitrogen, and sulfur, and the halogens in organic compounds by the methods of ter Meulen and Heslinga are reviewed by Guzman (*33*).

FUNCTIONAL GROUPS

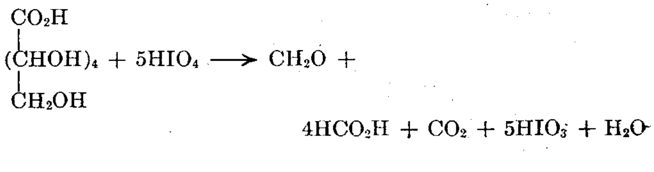
A very useful book has been published by Siggia (*89*) which describes many rapid volumetric methods for quantitative estimation of functional groups.

ACIDS

The amount of malic acid (*76*) in plant leaves (such as tobacco) can be determined by extraction with dilute sulfuric acid, neutralization with alkali, evaporation, and heating of the residue to 130° for 3 hours. This converts the malic acid to fumaric acid. The salts are dissolved in water, oxalic acid is precipitated with calcium acetate, and the fumarate is precipitated with mercurous nitrate as the mercurous fumarate. The mercurous ion in the latter is oxidized to the mercuric state and titrated with standard potassium thiocyanate as described by Stotz (*98*).

In order to obtain the total equivalents of lactic acid in its solutions which also contain lactyl–lactic acid and lactide it is recommended that the sample first be titrated to the neutral point, then saponified by an excess of alkali, and the excess alkali back-titrated with standard acid (*42*).

Gluconic acid may be determined by oxidation with periodic acid (*29*).



$$\begin{array}{l} CO_2H \\ | \\ (CHOH)_4 + 5HIO_4 \longrightarrow CH_2O + \\ | \\ CH_2OH \end{array}$$

$$4HCO_2H + CO_2 + 5HIO_3 + H_2O$$

The reaction requires about 2.5 hours at 36°. The titration is accomplished by adding bicarbonate and an excess of standard sodium arsenite and back-titrating with iodine.

When organic acids such as picric, maleic, or salicylic acid are titrated in the presence of Norite, the titration curves have the form typical of weak organic acids, indicating a buffering effect (*99*).

Phytic acid (*39*) may be determined by hydrolysis to inositol, then oxidation of the latter with an excess of periodic acid solution, followed by back-titration.

p-Aminosalicylic acid, its sodium salt, and *m*-aminophenol brominate quantitatively in aqueous solution with a bromide-bromate solution and this serves as a means for their estimation (*92*).

Guaiacol sulfonic acid (*16*) and quinosol have been determined by treating with acidified potassium bromide–bromate solution followed by iodometric titration of the excess bromine.

Ascorbic acid may be titrated in hot aqueous acid solution with a standard solution of ferric chloride and ammonium thiocyanate as indicator (*63*). Citric acid may be determined by oxidation with standard potassium permanganate in alkaline solution (*41*). The excess permanganate is titrated with standard sodium arsenite.

The decarboxylation of aromatic acids has been made the basis of a method for determining the carboxyl group in such compounds (*45*). The method is of value where the carboxyl group is too weak to be titrated or where the acid contains other groups besides the carboxyl which might make titration useless. The acid to be analyzed is heated in quinoline with basic cupric carbonate and the carbon dioxide evolved can either be measured volumetrically or absorbed on Ascarite and weighed. In cases where basic cupric carbonate is not an efficient catalyst—e.g., with 2,4-dichlorobenzoic acid—silver carbonate may be substituted. The method should be valuable in certain cases, even though the accuracy and precision are not high.

ACID CHLORIDES

A method for determining acid chlorides (*71*) makes use of a combination of several determinations and permits the calculation of free organic acid and free hydrochloric acid as well as the acid chloride.

The sample, *p*, is dissolved in purified dioxane and titrated with *a* ml. of 0.1 *N* alkali using bromothymol blue indicator. This same solution is then acidified with dilute nitric acid and a known excess of 0.1 *N* silver nitrate solution is added. A few drops of permanganate solution are added, the solution is diluted, and the excess silver nitrate is titrated with standard potassium thiocyanate solution. The milliliters of silver nitrate consumed are designated as *c*. A second sample, *p'*, is dissolved in dioxane and treated with aniline. After the reaction mixture is diluted with water it is titrated with *b* ml. of 0.1 *N* sodium hydroxide solution to a bromothymol blue end point. From the above data the following calculations are made. M = molecular weight of the acid chloride and m = molecular weight of the organic acid.

$$\%\ \text{acid chloride} = \left(\frac{a}{p} - \frac{b}{p'}\right) \times \frac{100\ M}{10.00}$$

$$\%\ \text{organic acid} = \left(\frac{b}{p'} - \frac{c}{p}\right) \times \frac{100\ m}{10.00}$$

$$\%\ \text{hydrochloric acid} = \left(\frac{c-a}{p} + \frac{b}{p'}\right) + \frac{36.5 \times 100}{10.00}$$

ACTIVE HYDROGEN

Lithium aluminum hydride (*116*) has been compared with methyl magnesium iodide as a reagent for detecting active hydrogen and reactive functional groups in thirteen organic compounds. The hydride was found to be superior in most cases, especially where hindered or enolizable groups are involved. It seems advisable in some cases to use both reagents, as this procedure often gives more information than either one alone. The number of moles of lithium aluminum hydride required for 1 mole of ketone is 0.25, of ester 0.50, of carboxylic acid 0.50 (carbonyl function only), and of nitrile 0.50.

The use of a butyl ether solution of lithium aluminum hydride as the reagent for determining active hydrogen and also reducible groups is reported by Hochstein (*44*). A useful design of apparatus for carrying out the determinations in a nitrogen atmosphere is described. Alcohols, phenols, and amines gave excellent results on active hydrogen determinations. Data are also given on various types of enolizable compounds and the amount of the reagent consumed by reducing action on numerous functional groups.

ALCOHOLS

The oxidation of the lower primary alcohols to the acids with an excess of 0.5 *N* potassium dichromate and varying amounts of sulfuric acid was studied (*75*). The extent of oxidation varied with the acidity, but none of the oxidations were quantitative. Despite this, it is suggested that the constituents of binary mixtures could be determined by using prescribed conditions and comparing the amount of potassium dichromate used by the mixture with that required by the individual alcohols.

ALDEHYDES

Some aromatic aldehydes may be determined by reaction with hydrazine to give the azine followed by titration

$$2\text{ArCHO} + \text{H}_2\text{NNH}_2 \longrightarrow \text{ArCH}{=}\text{N}{-}\text{N}{=}\text{CHAr} + 2\text{H}_2\text{O}$$

of the excess hydrazine (*31*).

Roche (*78*) has improved the sensitivity of the iodometric titration of aldehyde bisulfite in the presence of an alkaline buffer by careful titration to an end point which matches that of a standard reference solution of rosaniline and indigo carmine.

AMINES

Several amino sulfonamides have been determined by titration in anhydrous acetic acid (*102*). The titrating agent used is prepared from aqueous perchloric acid and sufficient acetic anhydride to react with the water present.

Basic nitrogen compounds in refined hydrocarbons (*111*) and in hydrocarbon oils and coal hydrogenation oils (*112*) have also been determined by titration with perchloric acid in glacial acetic acid with methyl violet as the indicator.

p-Phenylenediamine (*117*) may be determined by titrating a solution of the hydrochloride in acetic acid with a standard sodium nitrite solution using starch paper indicator.

In order to analyze crude and purified carbazole (*20*) an acetic acid–chlorobenzene solution of the sample is treated with a known excess of sodium nitrite. The carbazole forms a nitroso derivative and the excess nitrite is determined by decomposition to nitric oxide which is swept out by a stream of carbon dioxide and measured volumetrically in an azotometer. It is necessary to apply a correction for the reaction of some of the nitric oxide with the alkali of the azotometer and to assume that 3 moles of the excess nitrite give 2 moles of nitric oxide.

AMINO ACIDS

An extensive study of the action of sodium chlorite on amino acids has been reported (*107*). It appears that sodium hypochlorite is the active agent. The nitrogen in amino acids may be determined rapidly and accurately as ammonia by treatment with alkaline sodium hypochlorite. The evolved ammonia is absorbed in standard hydrochloric acid and back-titrated with alkali with methyl red–methylene blue indicator.

ESTER GROUPS

Studies on the determination of saponification numbers of fats and oils by the official method (*3*) have shown that variations in results may be due in part to the alcohol content of the solution during titration (*27*). When the water content of the final solution is above 65%, slightly higher saponification values are obtained. This is due to the increased hydrolysis of the soap under these conditions. Exposure of samples to the air, thus affording opportunity for absorption of carbon dioxide, appears to be of very slight importance as a factor contributing to variable results.

Acetate and butyrate groups in acetobutyrates of cellulose have been determined in the following way (*95*).

> The sample is hydrolyzed by digestion with 18 *N* sulfuric acid for 12 hours. Dibasic sodium phosphate and water are added to the digested sample in a Wurtz flask and the solution is distilled; the volume is kept constant by adding water through a separatory funnel until 250 ml. of distillate are collected. A 50-ml. aliquot of the distillate is titrated with standard sodium hydroxide solution. The remaining 200-ml. solution is distilled and two fractions of exactly 50 ml. each are collected and titrated. From the titration data the percentages of acetate and butyrate groups can be calculated.

SULFIDES AND DISULFIDES

A method for the determination of alkyl sulfides and disulfides (*90*) is based on the following reactions:

$$R_2S + Br_2 \longrightarrow R_2SBr_2 \xrightarrow{H_2O} R_2SO + 2HBr$$

$$RSSR + 5Br_2 + 4H_2O \longrightarrow 2RSO_2Br + 8HBr$$

The bromine required is generated by the addition of a standard bromate-bromide solution to an acid solution of the unknown. The end point is taken as the appearance of the first bromine coloration. The usual method of adding excess bromate-bromide and determining the excess reagent iodometrically cannot be used for disulfides because of substitution reactions which consume bromine and cause high results. The substitution reactions do not interfere as long as there is no excess bromine present. Mixtures of alkyl sulfides and disulfides can be determined by a combination of the bromine oxidation for both sulfide and disulfide and the method of Kolthoff (*55*) for disulfide alone. Best results using this combination are obtained on samples low in disulfide and high in sulfide. Thiols interfere unless they are determined separately and the results corrected.

SULFONAMIDES

A method for determining sulfanilamide (*50*) utilizes a standard solution of potassium permanganate to liberate a known amount of bromine from acidified potassium bromide solution. Addition of the sample of sulfanilamide results in consumption of some of the bromine due to nuclear bromination and the excess bromine is determined by addition of potassium iodide and titration with standard sodium thiosulfate solution.

It is claimed (*52*) that sulfonamides may be determined by adding a solution of potassium nitrate in sulfuric acid to a solution of the sulfonamide in concentrated sulfuric acid followed by measurement of the volume of nitrous oxide evolved.

p-Toluenesulfonamide in nickel baths has been determined (*93*) by the following procedure:

> Chlorine gas is bubbled through a portion of the solution to be analyzed. The white suspension or precipitate which forms (if the sulfonamide is present) is extracted with chloroform and evaporated to a small volume on a water bath and then dried completely in a vacuum desiccator at room temperature. The precipitate is dissolved in 30% potassium iodide and acetic acid, and titrated with standard thiosulfate. The method is applicable to other sulfonamides.

UNSATURATION

Styrene and some of its derivatives react quantitatively with mercuric acetate in methanol. The elements of methoxy mercuric acetate are added to the carbon-carbon double bond and one mole of acetic acid is liberated for each equivalent of double bond. This reaction has been made the basis of a rapid determination of such compounds (*60*). The ethylene compound to be analyzed is treated with an excess of mercuric acetate in methanol. After the reaction has been allowed to proceed for 10 to 15 minutes, sodium chloride is added, thus forming mercuric chloride. The acetic acid formed in the reaction can then be titrated with standard sodium hydroxide solution. Satisfactory results are obtained with styrene, divinyl and ethylvinyl benzene, allyl alcohol, crotyl alcohol, certain allyl ethers, and esters, certain vinyl ethers, and vinyl carbazole. Acrylonitrile, vinylpyridine, vinyl acetate, vinyl benzoate, and acrylate, methacrylate, itaconate, and maleate esters give poor results.

The method of analysis for aldehydes and some ketones using sodium sulfite solution followed by titration of the base liberated, is well known. It has now been pointed out (*115*) that maleic acid and its esters react with hot sodium bisulfite and this reaction can be used as a basis for their determination. Fumaric acid is stated not to react, but it can be isomerized to maleic acid and then determined. The procedure is adaptable to determining components of mixtures containing formaldehyde, crotonaldehyde, fumaric, and maleic acids and a nonreactive acid such as benzoic acid.

Small amounts of ethylene in nitrogen, oxygen, hydrogen, methane, and carbon monoxide have been satisfactorily determined by the following procedure (*97*).

> The gas to be analyzed is passed into an evacuated flask containing standard bromide-bromate solution, which is then acidified and agitated for 5 minutes. The bromine color is destroyed by the addition of sodium hydroxide solution. Potassium iodide and sulfuric acid are added and the liberated iodine is titrated with sodium thiosulfate solution. This method has been used with gases containing from 0.073 to 3.07% ethylene.

The bromine numbers of unsaturated acids, aldehydes, and ketones which have the unsaturation in the alpha-position (conjugated) may be determined by treating the sample with a standard solution of bromine in dry methanol saturated with potassium bromide (*72*). After standing in the dark for the necessary time, the excess bromine is determined by titration with a standard solution of anethole in methanol. The method was applied to rate studies on the formation of cinnamic aldehyde from benzaldehyde and acetaldehyde and on the isomerization of pseudoionone to α-ionone.

A solution of potassium bromate and bromide in the minimum amount of water and then diluted with pyridine and glacial acetic acid is recommended for the titration of double bonds (*14*).

MISCELLANEOUS METHODS

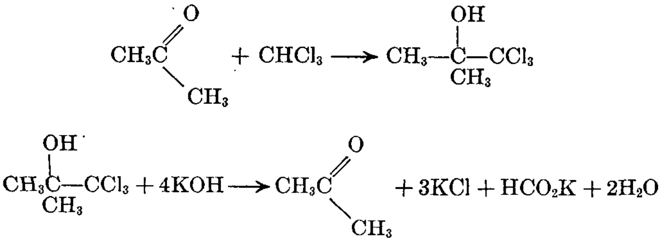
CHLORO COMPOUNDS

A procedure and an equation for the calculation of the *p,p'*-DDT content of commercial mixtures of the *o,p*- and *p,p'*-isomers have been worked out (*105*), which depend on differences in the rates of dehydrohalogenation by alcoholic potassium hydroxide.

Commercial products containing hexachlorocyclohexane (*77*) are analyzed for the latter by hydrolysis with alcoholic potassium hydroxide followed by a Volhard titration of the chloride ion.

The chlorine in 9-chloroacridines (*80*) is hydrolyzed by refluxing with 20% sulfuric acid and the resulting chloride ion is determined by the Volhard method.

The usual method of determining chloroform by alkaline hydrolysis to liberate chloride ion may give erroneous results because of the volatility of the chloroform. In a method developed by Pesez (*67*) this danger is avoided by carrying out the alkaline hydrolysis in the presence of acetone. In this way all of the chlorine can be liberated in less than one hour at room temperature. It is postulated that the hydrolysis goes according to the following equations.



Bromoform is hydrolyzed even more rapidly than chloroform, but dichloroethane, trichloroethylene, and tetrachloroethane are not appreciably hydrolyzed. The removal of one mole of hydrogen chloride from DDT is also speeded up by using acetone with potassium hydroxide (*68*). The reaction is complete at room temperature in about 10 minutes.

HETEROCYCLIC NITROGEN

The amounts of certain heterocyclic nitrogen compounds (*81*) can be estimated with an accuracy of about 2% by alkylating a sample with methyl sulfate and alkali. The quaternary salt is then treated with concentrated alkali and potassium permanganate, and steam-distilled. The amine which distills over is absorbed in an excess of standard acid and back-titrated with alkali. Volatile compounds are alkylated by heating with ethyl iodide in a sealed tube at 120° and then steam-distilled with alkaline permanganate.

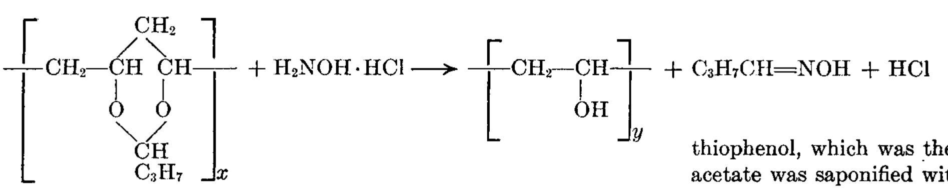
PHENOLS

The iodometric determination of certain phenols has been studied by Wooten (*113*).

The composition of mixtures of *o*- and *p*-cresols (*2*) can be estimated by conversion to cresoxyacetic acids by reaction with alkali and chloroacetic acid. The sodium salt of the para acid is less soluble and separates. Filtration and acidification yield the para acid whose purity can be estimated by melting point curves. The more soluble *o*-cresoxyacetic acid is obtained by acidification of the above filtrate.

POLYVINYL ACETALS

The number of butyral groups in polyvinyl butyral (*94*) is determined by refluxing the sample with alcoholic hydroxylamine hydrochloride for 1 to 1.5 hours.



Water is then added to dissolve any polyvinyl alcohol which may precipitate and the solution is titrated with standard alkali to a methyl orange end point. Polyvinylformal (*96*) is determined by hydrolysis with 20% sulfuric acid followed by steam distillation. The formaldehyde is determined volumetrically by treatment with hydroxylamine hydrochloride and titration of the hydrochloric acid with standard alkali as above.

SUGARS

It has been shown that the Munson and Walker method for determining lactose alone or in the presence of sucrose is unreliable when the copper value of 5.0 ml. of solution exceeds 200 mg. (*36*).

A very thorough reinvestigation (*40*) of the Shaffer-Hartmann-Somogyi method for determining reducing sugars has led to a modified procedure which gives more easily reproducible results. Data are given on the yields of cuprous oxide from fourteen aldoses and two ketoses and the results are correlated with the differences in configuration and structure of the sugars.

WATER

A modification (*86*) of the Karl Fischer reagent has been worked out so that it is no longer necessary to restandardize the reagent so frequently. Two solutions are used instead of the customary one. The sample is dissolved or suspended in one solution which consists of pyridine, sulfur dioxide, and methanol and then titrated with a solution of iodine in methanol. The precision of this method is of the same order of magnitude as the original method.

A method for the determination of water (*69*) in organic solvents is based on the following reaction of lead tetraacetate.

$$Pb(OAc)_4 + 2H_2O \longrightarrow PbO_2 + 4HOAc$$

The lead dioxide thus formed is determined iodometrically. Good precision is obtained on samples of nonhydroxylic solvents of 0.5 to 10 ml. containing 1 to 20 mg. of water. The success of the method depends partly upon the preparation of pure dry lead tetraacetate.

MIXTURES

The composition of ternary mixtures of toluene, *n*-butyl alcohol, and water may be determined (*87*) by titration of a known volume with water until a second phase just appears. From this titration and the refractive index of the predominating phase the composition can be calculated from known data on the ternary.

A rather complete set of volumetric procedures for analyzing technical xanthates for xanthate content and also amounts of sulfide, trithiocarbonate, sulfite, sulfate, and carbonate has been described (*54*).

Procedures for the analysis of mixtures of mono-, di-, and tri-(β-chlorovinyl)chloroarsines (*74*) with arsenic trichloride have been devised using differences in the rates of hydrolysis with alkali. The components are titrated iodometrically or with standard bromate solutions.

Some rapid methods for the determination of thiophenol, diphenyl sulfide, and phenyl thiolacetate have been developed in connection with studies on the cleavage of benzyl phenyl sulfide by aluminum bromide and other acidic reagents (*38*). Thiophenol was determined by titration with alcoholic iodine solution. The reaction was much more rapid when a few drops of pyridine were added. Other methods tried were unsuitable in the presence of benzyl bromide and hydrogen bromide. Diphenyl sulfide was reduced with zinc and acetic acid to thiophenol, which was then titrated with iodine. Phenyl thiolacetate was saponified with 30% alcoholic potassium hydroxide solution. The resulting solution was acidified and analyzed for thiophenol. By acidifying the reaction after 20 minutes, by which time the ester was completely hydrolyzed, it was possible to make satisfactory determinations of phenyl thiolacetate in the presence of large amounts of disulfide. If the hydrolysis was allowed to continue for longer than 0.5 hour some cleavage of the disulfide to mercaptan occurred.

In a procedure for the determination of butadiene dimer (4-vinyl-1-cyclohexene) in 1,3-butadiene (*34*) the dimer is first separated from butadiene in special distillation apparatus (*48*). The residue containing the dimer is then brominated with a bromide-bromate solution and the excess bromine is determined iodometrically.

A combination of specific gravity determinations and titrations of diacetone alcohol has been used to give a very rapid method for the determination of mixtures of isopropyl alcohol, diacetone alcohol, and 2-methyl-2,4-pentanediol (*30*). A standard solution of hydroxylamine hydrochloride is neutralized with sodium hydroxide solution to a greenish blue bromophenol blue end point. The sample, containing diacetone alcohol, is then added, and the solution is thoroughly mixed and allowed to stand for several minutes. The solution is then titrated with standard alkali, and the color change is compared to that of a blank.

UNCLASSIFIED

In a procedure for the determination of dinitrotoluene in smokeless powder (*12*) the dinitrotoluene is extracted with ether and reduced with standard titanous chloride solution. When the reduction is carried out in the presence of an alkaline buffer such as sodium acetate or sodium citrate, it is almost instantaneous at room temperature and results in a saving of about one third in time and manipulations.

Nitroguanidine (*56*) is reduced by standard titanous chloride solution in 15 minutes in acid solution. Cyclotrimethylenetrinitramine (RDX) is reduced only to the extent of about 60% by titanous chloride, even on prolonged heating. Ferrous chloride gives only a negligible amount of reduction, but when titanous chloride plus a little ferrous chloride is used the reduction is 98 to 99% complete. Four equivalents of titanous chloride are required for each nitro group.

A distillation method has been worked out for the determination of ethyl phthalate in smokeless powders (*13*).

A sample of the powder is digested with 30% potassium hydroxide, whereby the nitrocellulose and nitroglycerin are decomposed, and the diethyl phthalate is saponified to yield potassium phthalate and ethyl alcohol. Hydrogen peroxide is added to oxidize aldehydes present; the ethyl alcohol is then removed by distillation and determined by oxidation with potassium dichromate. It is necessary to attach a bent thistle tube water trap to the top of the condenser during refluxing to prevent loss of alcohol. With samples containing ethyl centralite it is necessary to add sodium sulfate and filter the steam distillate to remove some ethyl centralite which steam-distills over. A rather large blank is obtained primarily because of oxidizable impurities in the inorganic materials. The method is claimed to be more rapid and more accurate than the method of Thames (*101*).

The analysis of methallyl chloride has been investigated along several lines (*25*). Neither hydrolysis with alcoholic potassium hydroxide nor oxidation with dichromate and sulfuric acid gives quantitative recovery of chlorine. Applications of the method of Rosenmund and Kuhnhenn (*79*) and of Kaufmann (*51*) for determining unsaturation have been found to give satisfactory methods for the determination of methallyl chloride. The methods of Hubl (*46*), Wijs (*108, 109*), and Hanus (*37*) were unsatisfactory.

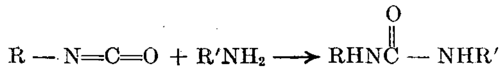
A convenient procedure consists of breaking a sealed ampoule containing a known amount of the methallyl chloride to be analyzed in a glass-stoppered flask containing an excess of standard Rosenmund and Kuhnhenn solution (pyridine sulfate dibromide). After a few minutes the contents of the flask are titrated iodometrically with a standard solution of thiosulfate.

Inasmuch as methallyl chloride is used as a fumigant, the above procedure was modified so that the substance can be determined as a vapor. In this modification a mixture of air and vapor is bubbled through the reagent. The reagent is volatile and so bromine might be lost during the bubbling. To avoid this loss the escaping bromine is collected in a bubbler containing a 2 *N* solution of sodium hydroxide and hydrogen peroxide. The following reaction takes place and the sodium bromide formed can be determined by the Volhard method.

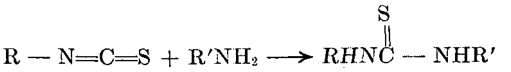
$$Br_2 + 2NaOH + H_2O_2 \longrightarrow 2NaBr + 2H_2O + O_2$$

Procedures have been devised for determining the amount of methallyl chloride in fumigated grain.

A method for the determination of organic isocyanates and isothiocyanates is based (*91*) on the following reactions:



$$R - N{=}C{=}O + R'NH_2 \longrightarrow RHN\overset{\overset{\displaystyle O}{\|}}{C} - NHR'$$



$$R - N{=}C{=}S + R'NH_2 \longrightarrow RHN\overset{\overset{\displaystyle S}{\|}}{C} - NHR'$$

By treating the isocyanate or isothiocyanate to be analyzed with a known amount of butyl amine solution and then determining the excess amine by titration with 0.1 *N* sulfuric acid, the isocyanate or isothiocyanate can be determined quantitatively. Because isocyanates and isothiocyanates react with water it is necessary to carry out the first step in dioxane. The butyl amine is dissolved in dioxane which has been dried over potassium hydroxide. A blank is run on the butyl amine solution each time it is used. Acidic or basic impurities in the sample to be analyzed interfere. Aromatic isocyanates and isothiocyanates react immediately with the butyl amine solution, but aliphatic compounds should be allowed to react for 45 minutes to ensure complete reaction.

The analysis of dimalone, the dimethyl ester of *cis*-3,6-endomethylene-4-tetrahydrophthalic acid, has been studied fairly extensively (*32*) because it is of importance as an insect repellent. Saponification is accurate for amounts down to about 30 mg. Special correction must be made in the presence of fixatives such as chlorinated paraffins. A bromination procedure is rapid and accurate for amounts as small as 25 mg. and is subject to only slight interference. Determination of the methoxyl groups is accurate with amounts down to 10 mg. but blank corrections and special apparatus are necessary. A colorimetric method can be used for approximation of small amounts down to about 2.5 mg. and is not subject to interference from pure-finish or sized cloth or from fixatives.

In a new method (*58*) for determining carbon monoxide in air the carbon monoxide is oxidized to carbon dioxide by passage over the catalyst hopcalite. In a modification of a previously described method (*21*) the resulting carbon dioxide is absorbed in excess barium hydroxide solution and the excess alkali is determined by titration with a standard solution of oxalic acid. A source of difficulty was the attack of the acid on the finely divided barium carbonate suspension when titration was carried to the phenolphthalein end point. Titration to the thymophthalein end point keeps the alkalinity above the point at which barium carbonate is attacked, but at the low acid concentration employed, the thymolphthalein end point is difficult to detect. It therefore seems easier to use phenolphthalein and apply a small correction.

The following procedure, designed for estimating tetraethyl pyrophosphate in insecticides which are phosphate ester mixtures (*24*), avoids the quantitative distillation of unstable tetraethyl pyrophosphate used in other procedures (*35*).

The esters are selectively hydrolyzed in aqueous sodium chloride solution under carefully controlled conditions. The higher pyrophosphates are completely hydrolyzed while hydrolysis of tetraethyl pyrophosphate is very slight and of a predictable amount. The unhydrolyzed pyrophosphate ester along with triethyl phosphate and a little diethyl acid phosphate is extracted with benzene. After neutralization of the diethyl acid phosphate, an excess of standard sodium hydroxide is added to hydrolyze the pyrophosphate without hydrolyzing the triethyl phosphate. The amount of tetraethyl pyrophosphate present can be determined from the amount of alkali consumed. All but 2.2% of the pyrophosphate present in the original sample is recovered in the analysis. The loss is due partly to hydrolysis and partly to incomplete extraction. It is corrected for in the calculations. The precision is ±0.5% of the tetraethylpyrophosphate content. A comparison of the method with the bioassay method has been made.

Absorption on an Amberlite resin is used in a new procedure for estimating tetraethylpyrophosphate in the technical grade product or in hexaethyl tetraphosphate (*114*). The sample is dissolved in 25% aqueous acetone. The ethyl polyphosphates are hydrolyzed rapidly to acidic products which are then removed by the acid-absorbing resin, Amberlite IR-4B. The tetraethylpyrophosphate is not hydrolyzed to any extent and remains in solution. It is later hydrolyzed to diethyl orthophosphate by an excess of standard base. This excess can be determined by titration with standard acid, using methyl red indicator, or by titrating to a pH of 6.0 using the glass electrode assembly.

The TAPPI standard method for the determination of pentosans in cellulose has been modified and shortened (*100*). The abbreviated method gives results comparing favorably with the TAPPI method.

The addition of alkanethiols and thiophenols to acrylonitrile has been used as the basis for a determination of the latter (*7*). An excess of a primary thiol, such as dodecanethiol, is added to the sample in the presence of a basic catalyst. After the solution has stood for a few minutes it is acidified and titrated with iodine solution to determine the excess dodecanethiol. The excess may also be determined amperometrically with silver nitrate. Either alcoholic potassium hydroxide or Triton B may be used as the basic catalyst. If the reaction time is long, some error is caused by oxidation of the thiol, but this can be corrected for by making proportional blank corrections. Another source of error, cyanoethylation of the alcohol solvent, is unimportant if the sample and thiol are mixed before the catalyst is added. The method is also applicable, with slight modifications, to the determination of α,β-unsaturated aldehydes and esters. The salts of unsaturated acids do not react. Unsaturated ketones do not react quantitatively.

The use of a liquid zinc amalgam (*62*) is advocated (*59*) as a reagent for the quantitative determination of aromatic nitro groups. The zinc amalgam rapidly reduces the nitro compound to an amino compound, which is then determined by bromination and titration of the excess bromine with thiosulfate.

One gram of nitrobenzene (accurately weighed) is reduced by shaking with 20 ml. of 2% zinc amalgam and 50 ml. of 4 *N* hydrochloric acid. The reaction mixture is diluted to 250 ml. in a volumetric flask. A 50-ml. aliquot of the solution is treated with 1 gram of potassium bromide, 50 ml. 0.2 *N* potassium bromate solution, and 10 ml. of concentrated hydrochloric acid, followed by 10 ml. of 40% potassium iodide solution. The mixture is allowed to stand for 15 minutes with occasional shaking and then titrated with standard thiosulfate solution. In the determination of solids, the reduction is speeded up by dissolving the sample in 10 ml. of methanol and precipitating it in a finely divided state by the addition of the hydrochloric acid. Suitable variations of the above procedure have been used for the analysis of *m*-dinitrobenzene, nitrobenzoic acids, nitrocinnamic acids, nitrophenols, nitrotoluenes, and nitrobenzene sulfonic acid.

Various carbazole sulfonic acids have been determined by precipitation with benzidine (*9*, *10*). The benzidine precipitate is dissolved in water and titrated with standard alkali to a phenolphthalein end point. Sulfuric acid must be removed by precipitation with barium chloride prior to the precipitation with benzidine.

To determine formaldehyde in urea resins, the resin is decomposed by treatment with sodium hydroxide and hydrogen peroxide (*19*). The formaldehyde is thus converted to formic acid, which is removed by steam distillation after acidification of the decomposition mixture with sulfuric acid. The formic acid in the distillate is determined by titration with standard alkali.

Sodium alkyl sulfates and related compounds may be determined by titration with a solution of cetyl pyridinium bromide (*28*). Chloroform and a little acid methylene blue are added to the solution to be analyzed. The blue color is concentrated in the chloroform layer at first, but as an equivalent amount of cetyl pyridinium bromide is added the two layers become the same color. If the equivalence point is exceeded the chloroform layer becomes colorless. Results are reproducible to ±0.5 to ±1.0% and the accuracy is good when the carbon chain of the sodium alkyl sulfate contains eight or more carbons. Saltor and Alexander (*82*) have also reported that anionic detergents may be titrated with cationic soaps using pinacyanol bromide as the indicator.

The oxygen numbers of oleic acid, linoleic acid, and tung oil may be determined (*53*) by treatment with an excess of perphthalic acid and the reaction followed iodometrically.

A review of the methods for determining metrazole and its behavior in the body has been contributed by Dister (*23*).

The use of ammoniacal nickel cyanide as an absorbent for benzene vapors has been shown to be unreliable in the presence of butadiene and butylenes (*4*). Allyl isothiocyanate (mustard oil) has been estimated (*15*) by treatment with an excess of acidified potassium bromide-bromate solution and the excess bromine determined by adding a known volume of standard potassium arsenite followed by back-titration with standard potassium bromate.

TETRAETHYLLEAD

The amount of tetraethyllead in gasoline may be determined (*57*) by adding solutions of magnesium perchlorate in ether and iodine monochloride in tetrachloroethane to the dried gasoline. The reaction

$$(C_2H_5)_4Pb + 4ICl \longrightarrow (C_2H_5)_2PbCl_2 + 2C_2H_5Cl + 2I_2$$

gives a precipitate of the diethyl lead dichloride and magnesium perchlorate. The precipitate is removed by filtration, dissolved in methanol, and titrated to a pink end point with a standard reagent composed of mercuric perchlorate and perchloric acid, using diphenylcarbazide as the indicator. The method may also be adapted to the determination of tetraethyllead in animal tissues and foods by using a preliminary extraction procedure.

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GAS ANALYSIS

LEONARD K. NASH, *Harvard University, Cambridge, Mass.*

ALTHOUGH, in recent years, the investigation of gas analytical methods has been prosecuted with great vigor and notable success, there is a paucity of texts and reviews which comprehend this far-flung pursuit in its entirety. One manual (*340*) provides a brief, elementary account of selected methods, and a more extensive treatment of classical methods of gas analysis can be found in a German text reprinted in this country (*17*). A monograph (*2*) in which the primary concern is with the analysis of gaseous fuels contains much material of general interest to the gas analyst. The use of microchemical methods in fuel gas analysis has been described (*192*); and comprehensive reviews of recent developments in the analysis of fuel gases (*112*) and petroleum gases (*171*) are available in other papers of this series. The problems and techniques involved in industrial air analysis receive thorough consideration in a recent book (*145*) and a major review paper (*286*); and equipment for the analysis of a special kind of industrial atmosphere—in mines—has been discussed (*26*). A variety of instrumental and volumetric analyzers receive brief consideration in several review articles on the analysis of industrial gases and atmospheres (*62, 109, 173, 231*).

Several of the more highly specialized types of gas analysis represent distinct, essentially independent units of analytical investigation. These methods have all received thorough reviews in other papers of this series, and elsewhere, so that any detailed examination in the present paper of their applications and capabilities would be supererogatory.

Distillation processes form one of these special domains. A general review is available (*254*), and a brief discussion of low-temperature analytical distillations (*138*) may be of interest. Recent developments of distillation apparatus and procedures (*33, 90, 291*) are of particular significance to the gas analyst.

The application of infrared methods, particularly in hydrocarbon analysis, has undergone a phenomenal expansion which is well mirrored in recent review papers (*14, 15, 48*). The usefulness of ultraviolet spectrophotometry (*255*) and Raman spectroscopy (*38*) in gas analysis has been indicated, and recent exploratory investigations (*39, 355*) give some promise of a fruitful development of gas analytical procedures based on x-ray spectrophotometry. To run the spectral gamut, it has been suggested that microwave spectrophotometry constitutes a potential analytical tool of enormous, though currently unrealized, capabilities (*69*). It could be applied only to strongly dipolar molecules of medium size, but could be made extremely sensitive and free of the ambiguities associated with overlapping absorption bands.

One of the most arresting recent developments in gas analysis has been the extraordinary incursion into the realm of practical analysis, made by mass spectrometry. Comprehensive reviews of the present status of analytical mass spectrometry have been presented (*32, 48, 136*), and extensive listings of cracking patterns, notably those of hydrocarbons, are available (*211, 248*). The manifold applications of mass spectrometry to gas analysis will not be considered here, but this instrument poses a real challenge to the older methods of gas analysis, and one that may well stimulate efforts to improve these methods.

That there is room for such improvement can be seen from "our first clear picture of gas analysis in this country"—provided by the results of cooperative investigation, in a large number of laboratories using various modifications of standard gas volumetric methods, of samples of a carbureted water gas (*277*) and a typical natural gas (*278*). When, in another cooperative project, the sample of natural gas was subjected to mass spectrometric analysis in many different laboratories, the results were clearly superior in their general concordance (*276*). Whether this is due to the inherent superiority of the mass spectrometric technique, or simply to the greater degree of standardization in its application, is not yet entirely clear. However, one more cooperative investigation—in this case, of mixtures of light hydrocarbon gases (*299*)—provides an appraisal of the relative capabilities, as applied to this analysis, of various volumetric, distillation, spectrophotometric, and mass spectrometric determinations, in which chemical volumetric methods are not highly rated.

The association of analytical mass spectrometry with various auxiliary operations, such as distillation, adsorption, hydrogenation, and selective chemical reaction (*191, 282*), should greatly enhance its powers by ameliorating some of its current shortcomings—such as its lesser sensitivity as applied to the analysis of isomeric olefins and its tendency to detect spurious traces. Coordinated analysis with both infrared and mass spectrometric methods should be particularly advantageous, for these two methods are largely complementary in their strengths and weaknesses (*197*). Mass spectrometry will present an increasingly formidable threat to many other techniques now in use.

GENERAL METHODS AND EQUIPMENT

SAMPLING

It is often much more difficult to secure a representative sample of a gaseous mixture than to analyze the sample so obtained. Even so "simple" a case as the procurement of a representative specimen of (contaminated) atmospheric air is not without its difficulties (*6, 119, 286*). Procedures for sampling the gases accumulated in the headspace of sealed tin containers (*53, 318*) and in sealed drug ampoules (*167, 304*) have been described. Gases dissolved in liquids can be extracted by warming under diminished pressure (*18*). A general technique which facilitates the extraction of gases from solution in highly volatile liquids—e.g. ether—has been developed (*190*). A manometric system of high precision, related to the Van Slyke apparatus, is well suited for the extraction (and analysis) of small volumes of gas from liquid solutions (*281*).

To secure a representative specimen of the gases dissolved in, and/or combined with, metals and alloys, high temperature vacuum extraction equipment may be used (*150, 184*). Combined vacuum fusion and ionic bombardment methods have been applied in the investigation of the gases in aluminum (*144, 203*); but a careful comparative study of various methods for the extraction of the gases in magnesium indicates that, at least in its present state of development, the ionic bombardment procedure is not fully satisfactory (*29*). The determination of the gases in copper alloys has been discussed (*88*); and various methods for the extraction and analysis of the gases in steel—with particular emphasis on hydrogen—are described in a group of papers on this topic (*325*). Low temperature gas extraction, based on use of chemical reagents, has been used in a study of the gases present as such in meteoritic and terrestrial steels (*210*).

When a specimen of a waste or process gas must be taken from a conduit in which it exists at comparatively high pressures, into an instrumental analyzer which functions at extremely low pressures, the sampling procedure and equipment must be designed to avoid an effusive separation and concentration of the gaseous components. Leak systems especially devised to secure representative specimens for mass spectrometric analysis (*137, 214, 215*) should be generally useful in sampling for any other low pressure instrumental analyzer.

The use of commercial infrared equipment to follow, in model experiments, the turbulent mixing of two gas streams (*61*) will make it possible to devise better procedures for securing representative specimens from composite streams.

A portable sampling-aspirator bottle can be used repeatedly without refilling (*302*); and various automatically operating fluid-displacement gasometers may be used to obtain gas samples that represent the time- (or volume-) integrated average composition of a streaming gas (*55, 243, 252, 315*).

Systems in which large volumes of gas are passed through an analytical train often require the use of auxiliary apparatus for the measurement of the total volume of the sample taken. The wet gas meter is customarily used for this purpose, and recent improvements in this equipment have made it capable of volumetric measurements accurate to a very few tenths of 1% (*4, 220*). A brief commentary on the present status of dry gas meters is also available (*7*). An indirect determination of sample volume is also possible, if the gas flow is fixed at a steady rate and the duration of the sampling period is measured. The rotameter may be adapted to use as a flow controlling instrument, by associating it with a control system that maintains the float at a predetermined level (*84*). Alternatively, gas flow rates from 0.002 to 2 cubic feet per minute can be established with an accuracy of a few tenths of 1% by the use of a series of critical flow orifices. To a first approximation the discharge coefficients for different gases are in accord with the theoretically predicted values; so extensive recalibration for each new gaseous mixture is probably unnecessary (*4*).

The problems involved in the sampling of liquefied petroleum gases have received attention (*79, 187*). A representative gas sample can be secured from a four-stage operation: (1) The liquid specimen is made homogeneous, (2) a portion of the liquid is withdrawn without ebullition, (3) this sample is completely volatilized, and (4) the vapor so secured is made homogeneous. To avoid the serious selective losses of vapor which may occur when hydrocarbons are handled in contact with hydrocarbon stopcock greases, various glycerol- and glycol-base lubricants have been proposed (*225, 238*). Particularly attractive is a grease compounded of fluorinated hydrocarbons, which is said to maintain vacuum-tightness for months at a time and which dissolves neither hydrocarbon nor more strongly dipolar materials (*73*).

Several blending systems which contain no greased taps have been suggested for the preparation of the determinate hydrocarbon mixtures required for the calibration and verification of various analytical processes. Gravimetric measurements serve to define the percentage composition of mixtures made in a simple blending system in which mercury-sealed sintered disk valves are used (*317*). Alternatively, manometric measurements may be used to determine the quantities of the individual components (*50*), and using an elaborate system (*166*), hydrocarbon mixtures of sharply defined composition can be prepared.

ABSORPTION AND ADSORPTION

The profoundly different efficiencies of various scrubbers, in the removal of trace components from streaming gases, are well illustrated by recent work on the determination of methanol vapor in air (*250*). With flow rates of 1 to 3 liters per minute and methanol concentrations of 200 to 400 p.p.m. the collection efficiency was 92% for a midget impinger, 91 to 96% for sintered-glass scrubbers, and 37 to 87% for scrubbing towers of various designs. Theoretical and experimental studies of the efficiency of recovery in a number of specific systems, and with scrubbers of different designs, have been completed (*128, 160–163*).

Adsorption processes may play a major role in gas analysis in relation to the use of upstream guard tubes, to remove interfering components; the concentration of trace components by adsorption; and chromatographic separation of closely related materials. The concentration of traces of halogenated organic compounds in contaminated air, by adsorption on activated charcoal (*72*) and on silica gel (*95*), is a process whose efficiency varies widely from case to case. However, because of its great generality, this technique is potentially very powerful.

The equilibrium adsorption characteristics of a large number of specific systems are well known (*47, 168*). Thermodynamic factors in adsorption processes have been discussed (*43*), and there has been a considerable amount of war-stimulated experimental and theoretical work on the kinetics of adsorption of trace components in packed columns—particularly those charged with charcoal (*71, 81, 134*). A review of some of this work is available (*156*).

Theoretical and experimental studies of the separation of the components of streaming gases during their flow through adsorptive masses indicate the great capabilities of this technique (*70, 348*). Thus it is possible to secure a clean separation of an equimolar mixture of propane and propylene by a single passage through a pretreated bauxite adsorbent. The gas interferometer has proved a valuable auxiliary tool in the adsorption analysis of hydrocarbons (*256*), and an interesting application of chromatographic techniques to the analysis of light hydrocarbon mixtures has been described (*328*). A theoretical and experimental study of adsorption processes, as applied to analysis for traces of helium and neon in air, is available (*116*).

VOLUMETRIC PROCEDURES

Macrovolumetric Analyzers. A series of discussions of the design and operation of most of the common macrovolumetric analyzers has been provided by Vint (*336*). A critical study of Orsat-type equipment has resulted in the suggestion of several possible improvements of current practice (*75*). Particular stress is laid on the aberrations arising from errors in the measurement of the carbon dioxide produced in combustions—a point to which attention has been drawn repeatedly in the past (*37*). Orsat-type equipment of unusually great flexibility and broad applicability has recently been described (*41*); and the application of the Orsat principle in the Duplex Mono recorder, for the automatic analysis of waste gases, has been discussed (*8*).

A simple auxiliary manometer, intended for use with the compensating burets frequently associated with Orsat analyzers, is said to permit closer equalization of the pressures in the buret and the compensator before connection is established between these two vessels (*5*). The calamities that result when a major pressure inequality exists at this stage of operations are thus avoided.

For industrial analyzers, where the use of mercury is inconvenient, various glycerol- and glycol-base leveling fluids have been recommended. However, it has been shown (*158*) that carbon dioxide is more freely soluble in these formulations than in an acidic aqueous solution of sodium sulfate which is, therefore, a better alternative in such an application.

It is suggested that the simple Hempel pipet may be used advantageously in place of the more complicated apparatus ordinarily employed for oxygen adsorption in the Haldane-Carpenter apparatus (*122*); and a modified Haldane apparatus with favorable operating characteristics has been described (*94*). Several improvements in the design and operation of the Bone-Wheeler equipment, which continues to maintain its popularity in Britain, have been indicated (*58, 92*).

A manometric method well adapted to the determination of small amounts of noncondensables in condensable gases or vapors has been developed (*152*). Using a grossly simplified manometric analyzer that requires only minimal quantities of mercury and reagents, it is claimed that acceptable results can be secured (*309*).

With a quick analyzer for respiratory gases, results accurate to 1% or better can be obtained in less than 5 minutes. The gas sample is taken in a syringe whose capacity has been fixed at, say, 5 ml., and is injected through a rubber tube directly into a reagent pipet, the upper portion of which terminates in a slender graduated tube in which the residual gas volume can be read. The difference between the original and final volumes represents the quantity of the adsorbable component (*263*). An improved syringe holder may be useful in such work (*74*). An alkaline solution of sodium (hyposulfite) and indigo carmine performs well as an oxygen adsorbent in this equipment (*303*).

Gooderham has continued the development of an interesting semiautomatic apparatus in which the volumetric measurements before and after each adsorption and combustion are made in a dynamic system, with sensitive soap-film flowmeters that operate without significant pressure drop (*120, 121*). Half a dozen components can be determined simultaneously with an accuracy comparable with that ordinarily attained with conventional volumetric equipment. In a related dynamic analyzer four matched capillaries are arranged in two parallel groups, to form a hydrodynamic resistance bridge containing a selective adsorbent placed between the two capillaries in one arm (*127*). Measurement of the pressure differential developed at analogous points in the two branches yields a continuous indication of the concentration of the adsorbable component.

Microvolumetric Analyzers. NORMAL PRESSURE. A simple horizontal microburet, intended for use in connection with the Blacet-Leighton analyzer, is joined to the rest of the apparatus through a ground joint, to facilitate cleaning operations (*174*). A refined yet compact micrometer buret, that also may be used with the Blacet-Leighton type analyzer, provides volumetric measurements with an accuracy of ca. 0.1% (*49*). Extensive volumetric calibration is unnecessary, and the apparatus contains a thermobarometer to compensate for changes in the ambient temperature and/or pressure.

With an apparatus based on use of the familiar Krogh mercury screw, adsorption analyses can be made with an accuracy of ca. 0.1% with a sample volume approximating 1 ml. (*56*). A 2-ml. sample is required in a simplified version of the Bonnier-Mangin apparatus which is capable of analyzing respiratory gases with an accuracy of a few tenths of 1% (*352*). With another apparatus for respiratory gas analysis the standard deviation of the results obtained with a 200-cu. mm. gas sample is somewhat less than 0.1% (*80*).

In a very simple Krogh-type apparatus, in which 10 cu. mm. of respiratory gas can be analyzed with an accuracy of ca. 0.1%, the volumetric measurements are made with the aid of a micrometer screw with which the confining fluid is driven through the buret

(*265*). In a very much more elaborate apparatus, in which a thermobarometer is incorporated, the volumetric measurements are again made with a micrometer, but a 0.5-ml. gas sample is required, and the results are accurate to ca. 0.015 volume % (*264*). An extremely refined apparatus and technique permit adsorptiometric analysis of a fraction of 1 cu. mm. of respiratory gas with an accuracy of a few tenths of 1% (*266*). Comparably small volumes of gas can be analyzed with very much simpler apparatus in which a length of thermometer capillary is used as a microburet, but the results are less accurate (*24*).

LOW PRESSURE. High vacuum gas analysis equipment is reviewed briefly in connection with a description of an improved apparatus of this type (*242*). The quantitative measurements are made with a McLeod gage, and the gases are circulated and collected with the aid of diffusion pumps. All separations are made by condensation and combustion procedures, and by outward and inward diffusion of hydrogen and oxygen through membranes of hot palladium and hot silver, respectively. Sufficient data are secured so that the determinations can be cross-checked, and with a sample volume of 5 cu. mm. the accuracy approximates 2%.

In a more elaborate apparatus, with much the same performance characteristics, the composition of the mixtures of nitrogen and inert gas, left after all other components have been removed, can be determined from the simultaneous readings of a McLeod and a thermocouple gage, or by measuring the rate at which the residual sample can be pumped out of the system (*153*). Other high vacuum microanalyzers show more variation in detail than in principle (*216, 237*).

The vapor pressures of various greases, waxes, and amalgams commonly used in high vacuum systems have been reported (*193*). A microdoser, for the introduction of minute amounts of gas into a vacuum system, has been described (*195*); and two palladium valves that are selectively permeable to hydrogen may be used in the introduction of this material (*293, 323*). When a diffusion pump is used as a collecting pump in these analytical systems it is necessary that the effective volume on the high pressure side of the jet be substantially independent of the (small) back pressure and of the rate of distillation in the pump. Such a condition is easily realized if a deflector-condenser is inserted at the foot of the mercury jet (*212*).

Low pressure analyzers in which chemical reagents are used tend to be somewhat simpler in structure and function. The Saunders-Taylor apparatus, in an improved form (*306*), yields satisfactory analyses of a few cubic millimeters of gas. Chemical reagents are used in another low pressure analyzer that requires considerably larger samples, but provides results accurate to a few tenths of 1% (*209*).

More specialized low pressure systems have been used for microanalysis of mixtures of condensables and noncondensables. The coordinated use of a cold trap and a McLeod gage serves to provide the necessary data (*91*); and in the Microvol the cold trap is made an integral part of a McLeod gage with a broad operating range (*260*). A few thousandths of 1% of carbon dioxide in a streaming gas can be determined by using a condensation trap to collect, and a vacuum system to measure, this component (*13, 357*).

INSTRUMENTAL PROCEDURES

Density. An exhaustive examination of various commercial specific gravity meters has been made at the Bureau of Standards (*288*). Very large differences in the capabilities of these instruments were observed; some were reliable to a few tenths of 1%, whereas others were occasionally in error by more than 1%.

The results secured with a microeffusiometer operating at low pressures and with minute samples of gases or vapors appear to be free of viscosity and specific heat effects; but it is doubtful that the equipment can yield accurate results with mixed gases (*207*). Another microeffusiometer that requires rather larger samples and is not entirely free of secondary effects is said to be applicable to the determination of isotopic mixtures (*22*). The interdiffusion of two gases through an orifice has been studied both theoretically and experimentally (*27*).

A novel laboratory densimeter consists of a hollow sphere supported by a flexible tube through which gas may be introduced into the globe. Observation of the equilibrium position of the sphere provides data for the calculation of densities said to be accurate to 0.1% (*314*).

Because of their comparatively high ratio of molecular weight to viscosity, traces of organic vapors in air can be determined with high sensitivity by the use of a viscosity-effusion meter (*132*).

A review of previous attempts to conduct gas analysis by measurements of sonic velocities—i.e., a function of molecular weight and the specific heat ratio—appears in connection with the description of a new instrument designed to provide the data requisite for such analysis (*66*). The velocity of sound in binary mixtures of hydrocarbon gases may be a simple function of the velocities in the individual components (*331*).

Thermal Conductivity. The application of thermal conductivity methods to gas analysis is the subject of a brief elementary review (*201*). A generalized thermal conductivity correlation for the gas state has been developed (*110*); and theoretical studies, supported by experimental data, have provided some estimate of the accuracy obtainable by the application of thermal conductivity methods to the analysis of several specific systems (*142, 143*). Major aberrations in thermal conductivity analyses made at pressures less than 100 mm. of mercury may result from small variations in the pressure. In a recently described thermal conductivity bridge these discrepancies are suppressed by an automatic compensatory device (*57*).

Applications of thermal conductivity analysis to respiratory gases (*23*) and argon-nitrogen mixtures (*44*) have been described. Although the extension of this method, to systems in which a plot of thermal conductivity against percentage composition displays a maximum, is superficially unpromising, there is only a small "blind spot" covering concentrations near the conductivity maximum, and results of satisfactory accuracy can be obtained over most of the concentration range. The system air–water vapor exhibits a conductivity maximum, but very satisfactory results can be obtained for all but a small range of concentrations. An automatic instrument particularly designed for this analysis was sensitive to changes of water concentration as small as 0.001% (*54*).

Because of the unique properties of hydrogen, mixtures of this gas with a melange of hydrocarbons can be treated as essentially binary mixtures with respect to their thermal conductivity. Analyses conducted under this simplifying assumption gave results that checked to better than 2% with the values derived from Orsat analysis for hydrogen (*344*). The unique behavior of hydrogen is also the basis of an indirect procedure for the determination of oxygen by thermal conductivity methods. The gas is mixed with hydrogen, and the difference of the conductivity of the mixture before and after the oxygen is catalytically converted to water, is almost entirely due to, and serves as a measure of, the diminution in the quantity of free hydrogen. From this diminution the quantity of oxygen can be calculated (*228*).

Although thermal conductivity methods are most generally useful as applied to binary mixtures, their applicability can be broadened. Thus some complex mixtures containing hydrogen can be treated as pseudo-binary mixtures (see above); and in general a grouping of components with similar conductometric properties provides a worth-while simplification (*244*). Furthermore, the coordinated use of several sets of thermal conductivity cells makes it possible to deal with mixtures containing as many as five components. The accuracy of the results so obtained, and the advantages and limitations of this general approach, have been examined (*83*). Finally, a procedure designed to take advantage of the distinct convective behavior of different gases may make possible a fairly general extension of thermal conductivity methods to ternary systems. Preliminary results secured with one such system are promising, but the general theory and the range of applicability of this method require further investigation (*200*).

Electromagnetic Properties. The development of an instrumental oxygen analyzer based upon the almost uniquely strong paramagnetism of this substance is one of the outstanding recent developments in gas analysis. In one form of this equipment the gas sample is held in an inhomogeneous magnetic field, in a chamber containing a heated wire that forms one arm of a Wheatstone bridge. The paramagnetic component is set in convective motion, owing to the temperature dependence of its magnetic susceptibility, and the magnitude of this convective cooling is inferred from the change of resistance of the hot wire. Instruments of this pattern (*87*, *246*) are capable of meeting most exacting specifications as to both accuracy and speed of response.

An alternative method of assaying gaseous paramagnetism is through a measurement of the rotation of a delicate torsion system which supports a small quartz test body in the gas specimen, which is enclosed within a strong inhomogeneous magnetic field (*223*). Other methods for the estimation of gaseous paramagnetism have also been described (*139*, *213*).

An instrument, which measures the convective cooling produced when the gas sample is held around a hot resistance wire and in an inhomogeneous electric field, has been used to explore the feasibility of an analytical method based on measurement of the concentration of paraelectric components of a gas mixture (*268*).

Other Properties. A general discussion of the classical method of determining traces of combustibles in air, by measuring the temperature rise accompanying the combustion that occurs when the sample is led over a catalytic surface, has been presented in connection with a description of an instrument of this kind developed for the British Admiralty (*235*). A generalization of this type of equipment is possible. Thus if a mixture of methanol vapor and air is conducted over a platinum wire which forms one arm of a Wheatstone bridge, the heat of combustion maintains the wire at a steady elevated temperature, and the bridge may be balanced under these conditions. However, if the air contains traces of a catalyst poison or combustion inhibitor (such as carbon monoxide, hydrocyanic acid, etc.) the oxidation is partially suppressed, the temperature of the wire falls, and an alarm can be actuated as the bridge goes out of balance (*30*). Even greater extension of this principle is possible, inasmuch as small quantities of any substance(s) which inhibit any exothermic catalytic reaction—e.g., enzymatic fermentation—can be detected by the changes they induce in the caloric output of the reaction mixture (*229*).

The calibration, use, and usefulness of the enormously sensitive gas interferometer have been discussed at length (*297*).

Proximate analysis of gases may be based on measurement of their ionizability (*147*, *194*), on a measurement of the mobilities of gaseous ions (*353*), or by measuring the frequency of occurrence of, and the energetic losses involved in, inelastic collisions suffered by the electrons of a monoenergetic beam that is impinged upon the gas sample (*135*).

SPECIAL METHODS FOR SPECIFIC MATERIALS

OXYGEN

The oxygen content of respiratory gases may be determined by thermal conductivity methods (*23*). An indirect determination of oxygen by thermal conductivity methods may be made both sensitive and accurate by basing the measurement on the unique conductivity behavior of hydrogen. Hydrogen is added to the test gas and the conductivity of the mixture is determined with reference to the same gas after it has been passed through a combustion chamber in which the only reaction is that of hydrogen with oxygen (*228*). The difference of the conductivities so recorded is primarily a measure of the diminution of the hydrogen content and of the oxygen content of the original gas.

The discovery that the chemisorption of oxygen on silver-plated glass wool produces considerable changes in the electrical conductivity of a tightly packed mass of the wool (*329*) may ultimately lead to an oxygen determination based on this phenomenon. The effect is reversible and is shown by few gases other than oxygen.

A classical determination of low concentrations of oxygen in streaming gases is based on measurement of the temperature rise produced when the gas, mixed with hydrogen, is passed over a combustion catalyst. If a thermocouple is used—the cold junction upstream, and the hot junction downstream, from the catalyst—an instrument largely independent of changes in the ambient temperature, and capable of continuously recording oxygen concentrations as low as 2 p.p.m., may be constructed (*59*). Instead of measuring the temperature rise accompanying the combustion, the water so formed may be taken as a measure of the oxygen content of the original gas. For example, the ammonia obtained by reaction of the water with hot (100° C.) magnesium nitride may be determined titrimetrically (*218*), or the water may be determined directly, by a volumetric or manometric measurement of the contraction accompanying its condensation (*111*).

Another possibility is the stoichiometric exchange of oxygen (and/or water and other oxygenous compounds) for carbon monoxide, by passing the test gas over charcoal at 1000° C. The carbon monoxide is then oxidized to the dioxide, which may be determined electroconductometrically after adsorption by a barium hydroxide solution (*285*). A determination of the oxygen content of a gas based on a photoelectric measurement of the relative transmissions of two samples of the test gas—to one of which a measured excess of nitric oxide has been added—has been patented (*131*).

In a novel determination of small amounts of oxygen in gases which do not contain any other oxidizing or reducing components the oxygen is concentrated by passing a measured volume of the gas over hot, freshly reduced, copperized silica gel (*77*). The analysis is concluded by a gas volumetric determination of the copper oxide so formed, in terms of the diminution in volume suffered by a measured sample of hydrogen when it is repeatedly circulated through the hot collecting tube.

If the oxygen is brought into a solution phase, a wide variety of methods for its determination in this condition are available. Polarographic measurement of the oxygen content of a solution that has been equilibrated with the test gas is satisfactory (*356*). Careful cell design aids in reducing aberrations due to secondary (stirring) effects which vary with the rate of gas flow (*20*). A more delicate, though less specific, determination of dissolved oxygen depends on a measurement of the applied potential at which the apparent charging current of a dropping mercury electrode becomes zero (*164*). Even minute concentrations of oxygen produce a major shift in this potential.

The classical Winkler method for the determination of dissolved oxygen has been applied to the determination of gas-phase oxygen by several investigators. A simple procedure employing a Bunte buret is possible (*169*), but much more delicate techniques have been developed. Gaseous oxygen in concentrations of 0.0001 to 0.1% may be evaluated with considerable accuracy by a relatively elaborate procedure (*354*). For the higher concentrations a titrimetric measurement is employed; for the very low concentrations a spectrophotometric determination of the starch-iodine complex present may be preferable. A very careful examination (*12*) of the spectrophotometrically concluded Winkler method has recently received full confirmation (*232*).

Very small concentrations of gaseous oxygen may be measured by shaking a determinate volume of the gas with freshly precipitated ferrous hydroxide. The ferric iron so produced is estimated colorimetrically, as the thiocyanate complex (*272*). For the determination of 0.001 to 5% oxygen in gaseous hydrocarbons a known volume of the gas may be allowed to react with metallic copper wetted with an ammonia–ammonium chloride solution. The oxidized copper is dissolved by the reagent and may be determined iodometrically (*330*). The excellence of this procedure has been confirmed recently (*76*). The apparatus and technique required are rather elaborate, and the same primary reaction has been made the basis of a greatly simplified determination, which is suitable for use in the field (*236*). In this instance the dissolved copper is determined colorimetrically.

A colorimetric procedure for the determination of gaseous oxygen in concentrations of ca. 1% depends on the progressive change of the color of indigo carmine produced by this gas (*182*). Oxygen concentrations as low as a few tenths of 1 p.p.m. may be estimated from the progressive decolorization of sodium anthrahydroquinone-β-sulfonate. The elaborate apparatus which is described (*35*) is capable of continuously and automatically recording the oxygen concentrations.

For the determination of major concentrations of oxygen by gas volumetric methods a number of oxygen adsorbents may be used, and a comparative study of several solution reagents has been reported (*312*). The use of solid iron sulfide as an oxygen adsorbent in volumetric analysis presents interesting possibilities. A careful examination of the adsorption reaction has been made (*126*), and it is reported (*120*) that the reagent performs very satisfactorily. Another promising reagent for volumetric oxygen determination may be a vanadous solution that is reported to adsorb, rapidly and completely, large volumes of oxygen from a streaming gas (*188*).

A very thorough study of the performance of a chromous solution as an oxygen adsorbent in volumetric analysis indicates that this old and occasionally maligned reagent is, when correctly handled, capable of giving excellent results (*305*). This conclusion is confirmed by one other investigation (*102*), but is contradicted by another publication (*358*) whose value each must assess for himself.

Major concentrations of oxygen are readily and semispecifically determinable from a measurement of the paramagnetic susceptibility of the gaseous mixture—for example (*223*).

Ozone. The bleaching action exercised on indigodisulfonic acid by ozone serves to determine a few parts per million of this component. Optimum conditions for sensitivity and specificity in this test have been described (*82*).

CARBON OXIDES

Carbon Monoxide. Largely because of the exigencies of war there has been a great deal of work on the estimation of trace quantities of carbon monoxide in air. A brief review of field methods for this determination is available (*199*); and an authoritative survey, based upon analyses of standard samples by a wide variety of methods and in a number of different laboratories, has been made (*279*). Results accurate to ca. 0.001% were not unusual. A number of selective reagents and reactions are available for this analysis:

Hopcalite. A brief account of some Hopcalite formulations has been presented (*67*); and an important study of the physical chemistry of the catalytic combustion on Hopcalite has been reported (*233*). With simple apparatus traces of carbon monoxide in air may be determined from the temperature rise engendered when this component burns over Hopcalite (*176*). Mercury-in-glass thermometers are used, and an accuracy of ca. 0.005% is secured over the concentration range 0.1 to 0.01% carbon monoxide. More elaborate equipment based on the same principle permits continuous recording of the carbon monoxide concentration (*177*). Instead of measuring the temperature rise accompanying the combustion, the determination may also be concluded by either a microgravimetric (*259*) or a titrimetric (*175*) measurement of the carbon dioxide produced.

Silver Oxide. The preparation and performance of activated silver oxide catalysts for the selective oxidation of carbon monoxide have been described (*31*). These catalysts, like Hopcalite, are effective at room temperature, but they enjoy a conspicuous advantage in that they are not deactivated by exposure to moisture.

The reaction of carbon monoxide with silver oxide catalysts appears to be accompanied by several side reactions which hinder the successful use of this reagent in gas volumetric determinations of substantial percentages of carbon monoxide (*313*).

Silver Permanganate. Measurement, with a thermocouple, of the temperature rise produced by the selective oxidation of carbon monoxide on silver permanganate provides analyses accurate to ca. 5 p.p.m. over a concentration range of 10 to 200 p.p.m. of carbon monoxide (*149*).

Mercuric Oxide. A titrimetric determination of the carbon dioxide produced by the selective oxidation of carbon monoxide over yellow mercuric oxide has been described (*245*). Because of its greater stability red mercuric oxide must be heated before it will produce selective oxidation of carbon monoxide; but the performance of this reagent is much more reproducible than that of yellow mercuric oxide. A laboratory procedure for the determination of trace quantities of carbon monoxide is based upon the weight loss suffered by a heated tube containing red mercuric oxide, through which a measured volume of the test gas is passed (*179*). The gravimetric factor is extremely favorable, as for each molecule of carbon monoxide entering the system one molecule of mercuric oxide is removed from the weighing tube. In a portable analyzer based on the same primary reaction the determination is concluded by a photometric determination of the blackening produced on a selenium sulfide test paper by the mercury vapor liberated in the catalytic oxidation of the carbon monoxide (*19*). This method is rapid and yields results accurate to ca. 10% with carbon monoxide concentrations ranging from a few parts per million to a few per cent.

Iodine Pentoxide. With the better grades of this reagent now available satisfactory results can be obtained, provided that adequate steps are taken to remove, by the use of upstream scrubbers, etc., the many materials, such as olefins, which interfere with the determination. In the absence of such precautions it has been shown that the values obtained with iodine pentoxide methods may be much too high (*146*). Vandaveer has suggested (*332*) some improvements of a procedure previously described by him. Spectrophotometric analysis of a solution of the iodine liberated by reaction of the pentoxide with carbon monoxide is said to provide results accurate to 3 to 10% over the concentration range of 0.001 to 0.2% carbon monoxide (*287*). In connection with a review of methods for the determination of carbon monoxide in fuel gases, a new portable apparatus which yields results accurate to ca. 5% for carbon monoxide concentrations ranging from 10 p.p.m. to 0.1% is described (*283*).

Palladous Salts. A number of formulations of palladium-bearing materials entering into selective reaction with carbon monoxide have been developed. A brief report has been (*270*) made on a determination in which the discoloration produced by carbon monoxide on a white test paper impregnated with palladium chloride is measured by reflectance spectrophotometry. Other test paper methods have also been proposed (*245, 337*). Alternatively, the discoloration produced by the test gas in an acetone solution of palladous chloride and phosphomolybdic acid may be measured with a photoelectric colorimeter (*234*). Or the metallic palladium produced by reaction of the carbon monoxide with a determinate solution of a palladium salt may be filtered off to permit a colorimetric measurement of the excess reagent (*124*).

As the result of a most exhaustive investigation of the usefulness of palladium-bearing reagents in carbon monoxide analysis, a verdict is rendered in favor of a test body consisting of silica gel impregnated with an acid solution of palladous sulfate and ammonium molybdate (*275*). For field work visual comparison of the discoloration of the test specimen with a card showing various standard discolorations is said to be adequate. In a closely related procedure discoloration is measured photoelectrically (*60*).

BLOOD. The semiselective and tenacious adsorption of carbon monoxide by blood provides a convenient concentration of traces of this gas in air. The determination may then be concluded by some standard method for the measurement of the carbon monoxide content of blood—e.g., the Van Slyke technique. Such an approach has certain very advantageous aspects (*269*). Several improvements in the traditional Van Slyke procedure are presented in connection with a review of the application of this method to the determination of the carbon monoxide content of blood (*334*). A microvolumetric Van Slyke apparatus with very favorable operating characteristics has been described (*346*).

An alternative technique, based on the use of a syringe analyzer, is said to be rapid, simple, and satisfactorily accurate (*267*). In a more highly refined, though somewhat cumbersome, technique both the Van Slyke manometric equipment and the syringe analyzer are employed (*257*).

In these methods the blood serves only as a collecting agent, and the determination is concluded microvolumetrically, after releasing the carbon monoxide from the blood. There are, of course, other possibilities. For example, the carbon monoxides may be evolved from the blood in a Conway microdiffusion cell, the other compartment of which holds a a palladium reagent with which the determination is completed colorimetrically (*124*).

CUPROUS SALTS. With a cuprous sulfate–2-naphthol reagent used in conjunction with standard Haldane-type volumetric equipment good results can be secured for carbon monoxide concentrations of the order of a few tenths of 1% (*25*). Hydrocarbons, particularly if unsaturated, should be removed prior to this determination.

The relatively complex state of combination of carbon monoxide adsorbed by various cuprous solutions has been the subject of several studies (*78, 86, 221*).

Other (combustion) methods which are essentially nonselective in their action have also been described. In a refined autometer for the continuous determination of carbon monoxide (and/or dioxide) (*45, 46*) determinate quantities of sample are intermittently injected into a steady stream of air. The mixture then passes to a copper oxide combustion tube and is finally scrubbed by a barium hydroxide solution which flows at a steady rate between the electrodes of an automatic recording conductivity bridge. The results are accurate over a wide range of (small) concentrations.

Traces of carbon monoxide in very rapidly flowing gas streams may be incompletely burned over the oxidic agents usually used for this combustion (*52*). More complete combustion was secured in the presence of excess oxygen and a hot bright-platinum catalyst, and the determination was concluded by a gravimetric measurement of the carbon dioxide produced. Using conventional high precision gas volumetric equipment, a painstaking study has been made (*37*) of the platinum-catalyzed combination of oxygen and carbon monoxide as a means of determining substantial concentrations of the latter component. When the determination is based on the total volume contraction and the oxygen consumption involved in the combustion, results accurate to a few hundredths of 1% can be secured. Less accurate results are obtained if the carbon dioxide produced is used as the basis of the calculation, largely because of the ready adsorption of this material by water, rubber, and/or tap lubricants.

Carbon Dioxide. The measurement of this component is usually grounded directly or indirectly on its acid properties, and the following methods for its determination assume the previous removal of all other acid gases. Electroconductometric evaluation of the changes produced in an alkaline solution by adsorption of carbon dioxide is used in an autometer for the accurate determination of low concentrations of this component (*45*). In a titrimetric method for carbon dioxide in natural gas a metered volume of the test gas is passed through a sintered-glass scrubber containing an aqueous solution of phenolphthalein, to which determinate quantities of aqueous barium hydroxide can be added from a buret attached to the scrubber (*28*). Minute traces of carbon dioxide in air may be determined with good accuracy by a photoelectric measurement of the extent of decolorization produced when a known volume of test gas is passed into a 0.0001 *N* sodium hydroxide solution containing phenolphthalein (*295*).

The carbon dioxide content of respiratory gases has been determined by a thermal conductivity method (*23*).

NITROGEN COMPOUNDS

Nitrous Oxide. To detect traces of nitrous oxide, oxygen is first removed from the sample and the nitrous oxide is then decomposed over hot platinum. The oxygen so liberated is determined colorimetrically with a test paper impregnated with alkaline pyrogallol (*262*). The test may be made even more delicate by previous condensation concentration of the nitrous oxide. The determination of major concentrations of nitrous oxide with commercial gas volumetric equipment, using either slow combustion or catalytic combustion techniques, is reported to be satisfactory (*204*).

Nitric Oxide and Nitrogen Dioxide. In the presence of excess air (or oxygen) nitric oxide is quantitatively converted to nitrogen dioxide (tetroxide), for which Griess-Ilosvay reagent provides a very delicate test. In a simple field test using this reagent all the analytical operations are carried out in a small syringe (*222*); and permanent colorimetric standards for this analysis can be prepared (*10*). A closely related procedure, in which traces of nitrogen dioxide in air are determined by adsorption on silica gel impregnated with diphenylamine, also relies on visual estimation of the discoloration produced (*99*). Using electrolytically generated oxygen, traces of nitric oxide in coal gas may be converted to nitrogen dioxide, which is then determined with Griess-Ilosvay reagent (*85*). The method is said to be potentially serviceable on a continuous recording basis. Alternatively, the nitric oxide may be oxidized in a bubbler charged with acid permanganate solution (*284*). Unless a separate determination is made of the nitrogen dioxide originally present as such, the analysis yields only the sum of the nitric oxide and nitrogen dioxide contents. Traces of nitric oxide in gases which do not contain olefins can be collected in a specially designed scrubber containing alkaline permanganate solution (*155*). The determination is concluded by assaying the nitrogen content of the solution with a micro-Devarda technique.

Traces of nitrogen dioxide may also be determined by photometry in the gas phase. A convenient monochromatic source is available in the sodium D doublet, which is strongly absorbed, and with tubes 1 to 2 meters long a sensitivity approaching 1 p.p.m. can be secured (*148*). An alternative photometric system is available (*289*). To analyze for both nitric oxide and nitrogen dioxide the absorption due to the nitrogen dioxide alone is measured first. The gas is then oxidized and again subjected to photometric examination. The nitric oxide is determined by difference (*183, 219*).

Major concentrations of nitric oxide in microsamples may be evaluated with Blacet and Leighton equipment. This component is removed from the gas phase by a bead of potassium hydroxide in the presence of excess oxygen (*290*). In an alternative process of somewhat more certain stoichiometry the nitric oxide is adsorbed by a bead impregnated with alkaline sodium sulfite (*170*). Even in the presence of olefins and acetylenes the results are accurate to a few tenths of 1%.

Other Nitrogen Compounds. For the determination of hydrogen cyanide in coke-oven gas the cyanide is collected in a scrubber and successively and stoichiometrically exchanged for thiocyanate, cyanogen bromide, and iodine—the latter being determined titrimetrically (*274*). This carefully developed procedure is equally useful for trace analysis and for the determination of larger proportions of hydrogen cyanide. Hydrogen cyanide may also be fixed (as a double salt) by a moist mixture of nickel and sodium carbonates, from which it is subsequently freed by acid distillation and determined by argentometric titration (*316*). A method for the detection of a fraction of a microgram of hydrogen cyanide per liter of gas is based upon the discoloration produced when the test gas is passed over silica gel impregnated with a cupric salt–benzidene reagent (*105*).

Traces of nitromethane vapor in air can be accurately determined by adsorbing the nitromethane in a dilute sulfuric acid solution, which is then subjected to polarographic examination (*351*).

SULFUR COMPOUNDS

Sulfur Dioxide. A delicate qualitative test for sulfur dioxide, based on the use of a fuchsin-formaldehyde reagent, has been studied extensively (*1, 123, 301*). In another spot test for this material a chain of inorganic redox reactions is involved (*21*).

Using a simple midget impinger technique, concentrations of sulfur dioxide as low as a few parts per million can be estimated from the rate of decolorization of a determinate amount of starch-iodine reagent (*224*). Other oxidizing or reducing gases interfere. Improvements of a previously described autometer for the determination of sulfur dioxide (or total sulfur compounds) in air have been suggested by Thomas *et al.* (*320*). In a closely related apparatus built around a new countercurrent scrubber, sulfur dioxide concentrations from a few parts per billion up to several per cent are measured in terms of the electroconductometric changes produced in the scrubber solution (*321*).

Precautions in the sampling of stack gases, and directions for the determination of their sulfur dioxide and total sulfur contents, have been described (*154*).

Sulfur Trioxide. Methods of analysis for small amounts of sulfur trioxide in stack gases that are relatively rich in sulfur dioxide have been much investigated, particularly by the British. If a metered volume of the gas is scrubbed with an alkaline solution containing glycerol, all the sulfur trioxide and some of the dioxide are retained. The glycerol inhibits oxidation of the sulfite, so that the sulfur dioxide (and oxygen) can subsequently be expelled from the solution by acidification and the passage of a stream of nitrogen. The sulfur trioxide, as sulfate, is then estimated by amperometric titration with lead (*11*). To determine both sulfur dioxide and trioxide, a metered volume of gas may be passed first through a scrubber containing pure distilled water and then through one charged with hydrogen peroxide solution. All the sulfur trioxide and a little of the dioxide are retained by the first bubbler; the balance of the dioxide is collected in the second bubbler (*114*). The rate of oxidation of the sulfur dioxide in the solution in the first bubbler is said to be very slow. This solution is titrated with alkali to a methyl red end point, from which the sulfur trioxide concentration is inferred. Hydrogen peroxide is then added and the end point is restored, the amount of alkali required serving as a measure of the sulfur dioxide retained in the first scrubber. This may then be added to the titrimetrically determined sulfur dioxide content of the second bubbler. If other acid gases may be present, a more elaborate titration procedure is required. In an autometer for the continuous determination of the sulfur trioxide content of stack gases, the sulfur trioxide is similarly adsorbed in distilled water, the solution is stripped of sulfur dioxide by a stream of inert gas, and the sulfur trioxide concentration is deduced from electroconductometric measurements of the scrubber solution (*115*).

Several major sources of error in the usual methods for sulfur trioxide analysis are avoided in a carefully developed procedure in which the sulfur trioxide is retained in a bubbler containing a water–isopropyl alcohol solution (*101*). The oxidation of the dioxide is almost completely inhibited in this solution. After aeration to expel the sulfur dioxide, the sulfate is determined gravimetrically. This analysis may be made more rapid by eliminating the aeration and the gravimetric determination (*63*). In the presence of isopropyl alcohol the turbidimetric estimation of sulfate is sensitive and accurate; and interference due to the presence of sulfite (sulfur dioxide) is inappreciable if the solution is made slightly acid.

Hydrogen Sulfide. To measure the hydrogen sulfide content of natural gas, the sample is passed at a metered rate through a scrubber containing starch solution to which a standard iodine solution may be added from a buret (*28*). The amount of iodine solution consumed by a given volume of the test gas (which is assumed to contain no other active reductants) gives the hydrogen sulfide content of the gas with an accuracy of a few hundredths of 1%. The determination of hydrogen sulfide by precipitation of cadmium sulfide and iodometric titration of the latter has been re-examined (*230*).

Traces of hydrogen sulfide may be estimated from the intensity of the stains which they develop on a lead acetate test surface (*319, 338*). An autometer for the evaluation of traces of hydrogen sulfide is based on a photometric measurement of the blue coloration produced by hydrogen sulfide in a sulfuric acid solution of ammonium molybdate (*296*). Two very delicate tests for traces of hydrogen sulfide have been described by Field and Oldach (*97*). In one the transmission of a suspension of bismuth sulfide is determined spectrophotometrically. The transmission appears to be a linear function of the sulfide concentration, and results accurate to 10% are secured with as little as 7 micrograms of hydrogen sulfide. The second method is somewhat less sensitive, but requires only the use of a visual comparator for the estimation of a suspension of cadmium sulfide. The presence of uranyl ion in the solution appears to improve the visual discrimination.

A somewhat simpler analysis for traces of hydrogen sulfide employs the methylene blue method (*159*). The hydrogen sulfide may be collected as zinc sulfide, which is then treated with ferric chloride and *p*-aminodimethylaniline. The methylene blue so produced is estimated spectrophotometrically, and the hydrogen sulfide concentration is estimated from a calibration curve (*103*).

Small amounts of hydrogen sulfide in carbide-generated acetylene are estimated after adsorption in a sodium hypochlorite solution. The sulfate may be determined by an indirect colorimetric procedure (*227*) or by conventional turbidimetric techniques (*36*). In the latter case a sensitivity of ca. 0.001% is secured.

Total Sulfur. To determine the total sulfur content of a gas, the sample can be pyrolyzed in the presence of excess oxygen and with or without a (platinum) catalyst. The sulfur oxides so produced are collected, converted to sulfate by wet oxidation, and then measured titrimetrically (*3, 96, 130, 141*).

Instead of pyrolyzing the sulfur compounds to oxides, it is often more convenient—for example, when dealing with gases rich in combustibles—to convert all the sulfurous material to hydrogen sulfide. If the test gas, mixed with at least a 30% excess of hydrogen, is passed over alumina at 900° C., the conversion of most other sulfur compounds to hydrogen sulfide is substantially complete, even when much organic material is present (*98*). The hydrogen sulfide so produced is collected in alkali and determined by any standard method (*130*). This otherwise desirable procedure presents difficulties as a field method and/or when much oxygen is present in the gas. Both these shortcomings are reduced if the sulfur compounds are first concentrated by drawing a measured volume of the test gas through silica gel. Most sulfur compounds are thereby adsorbed, though hydrogen sulfide and carbon oxysulfide are not retained, and must be separately determined by one of the several special methods available for them. In the laboratory the sulfur compounds are desorbed at 500° C., mixed with a stream of hydrogen, and passed through a quartz chip bed held at 1000° C. The hydrogen sulfide formed may be estimated by any standard method (*104*).

Individual Sulfur Compounds. Indirect determination of a number of individual sulfur compounds can be accomplished by measuring the total sulfur content of the test gas (see above) before and after the removal, with various selective adsorbents. of the individual sulfurous components (*129, 180, 247*).

The test gas is passed through progressively longer trains made up of the following component scrubbers, and the changes so produced in the total sulfur content are noted. Dilute hydrogen peroxide adsorbs the sulfur oxides; aqueous cadmium chloride is used to adsorb hydrogen sulfide and the mercaptans (thiols) from the remainder; 95% sulfuric acid followed by aqueous sodium carbonate provides for the further removal of the thiophene; and the residual gas, which should contain carbon disulfide and oxysulfide as its only sulfurous compounds, may be scrubbed with (*a*) piperidene-chlorobenzene solution, or (*b*) alcoholic potassium hydroxide, which remove both components, or with oil, which removes only the carbon disulfide. In case (*a*) colorimetric distinction of the carbon disulfide and oxysulfide is possible; in case (*b*) iodometric resolution is used. Hydrogen sulfide and mercaptans may be determined separately in the cadmium chloride solution by decomposing the mercaptides with dilute acid and then determining the cadmium sulfide iodometrically.

In a related procedure which does not involve the use of specific adsorbents, the progressive change in the total sulfur content produced by the equilibration of increasingly large volumes of the test gas with a known volume of an inert (oil) solvent is used as the basis for a qualitative and quantitative estimation of the sulfur compounds in the gas (*151, 217*). This method provides a powerful tool for the complete determination of individual sulfur compounds in particular, and it may be useful for similar analysis of closely related gases in general.

All these procedures are indirect and somewhat tedious. A simple colorimetric test for mercaptans has been described (*345*), and a direct spectrophotometric method for the simultaneous determination of thiophene, carbon disulfide, and carbon oxysulfide in producer gas has been developed (*34*). The gas is brought in contact with a piperidine-ethanol solution, and the light adsorption at three wave lengths, each of which is associated with an adsorption band characteristic for the adduct of one of the compounds named above, is measured. Mercaptans and hydrogen sulfide are also adsorbed by the solution, but do not interfere with the determination.

HALOGEN COMPOUNDS

The most popular approach to the determination of traces of halogen compounds (particularly halogenated hydrocarbons) in air has been through the pyrolysis of the test gas, usually in the presence of a platinum catalyst. The pyrolysis products may be determined conductometrically (*320*), colorimetrically (*324*), or turbidimetrically (*107, 261*); or by argentometric (*178, 258*), oxidimetric (*258, 349, 350*), or acidimetric (*118, 271*) titration. The accuracy and sensitivity vary greatly with the case in hand; but a sensitivity extending into the range of 10 to 50 p.p.m. or less, and an accuracy of 1 to 10%, are not unrepresentative. Preliminary concentration of the trace component(s) from a large volume of sample may help to increase the effective sensitivity. Such concentration is conveniently realized by selective adsorption (*72, 95*); or by passing the gas through an appropriate scrubber (*251, 343*). The concentrate can then be evaluated by any standard method of analysis.

The sampling and analysis of boron trifluoride have been investigated and a titrimetric procedure for the determination of boron trifluoride and silicon tetrafluoride in the presence of one another has been developed (*308*). A gas volumetric procedure for the same analysis has been based on the conversion of the boron trifluoride to acetyl fluoborate, followed by the separation of the latter from the much more volatile silicon tetrafluoride (*196*).

For the estimation of traces of chlorine in inert gas streams, the gas may be scrubbed with a dilute solution of hydrochloric acid, the changes in which are evaluated electroconductometrically (*165*). For the determination of hydrogen chloride in the presence of chlorine, all of the former and some of the latter are collected in a water scrubber. It is shown (*16*) that all the chlorine can be boiled off under a reflux condenser without undergoing any substantial amount of hydrolysis. No loss of hydrogen chloride occurs in this process, and the hydrochloric acid solution is then analyzed by acidimetric titration.

Small amounts of hydrogen fluoride in a gas stream may be evaluated in terms of the rate of decolorization of a test body impregnated with the zirconium-alizarin lake which is suspended in the gas (*186*). For the determination of substantial concentrations of fluorine in mixtures with air or nitrogen, the gas may be passed over hot sodium bromide, to procure a stoichiometric exchange of fluorine for bromine, and the latter is determined by photometric examination of the gas stream (*208*).

HYDROCARBONS

General Methods. A most important contribution to the volumetric analysis of hydrocarbons in particular, and gases in general, has been made by Brooks and co-workers (*41*), who describe an improved Orsat-type apparatus of great flexibility. Various adsorbent agents are examined, and several comprehensive patterns of analysis for complex mixtures of gases are proposed.

The replacement of the slow combustion pipet with a catalyst tube containing platinized silica gel has been suggested (*157*). The danger of explosion is reduced to a minimum, satisfactory fractional combustion is often possible, and the same catalyst may be used for analytical hydrogenation procedures. Clean fractionation is also said to be obtainable on platinized asbestos, provided that the temperature of the catalytic body is raised extremely slowly to its final value (*333*). A combustion element of copper oxide promoted with 1% iron oxide allows satisfactory fractionation in combustion, though in this respect it is not clearly superior to pure copper oxide. However, with the promoted oxide, methane can be burned more rapidly and/or at a lower temperature than with the pure oxide (*206*).

Traces of combustibles in air may be detected by the temperature rise produced when the gaseous mixture is passed over a catalytic element (*235*). The minute hydrocarbon content of subsoil air has been estimated from a titrimetric determination of the carbon dioxide produced when the gas sample is led over a heated platinum filament (*327*).

Saturated Hydrocarbons. For the determination of cyclopropane in the presence of propylene a selective hydrogenation procedure that works well with as little as 1 cc. (N.T.P.) of sample has been described (*64*). A selective adsorption procedure is also possible, as cyclopropane is not attacked by an acid mercuric sulfate reagent which adsorbs olefins rapidly. Cyclopropane can be determined in the residue from this treatment, by adsorption in 87% sulfuric acid (*42*).

Olefins. Some of the older reagents for the adsorption of ethylene have been re-examined (*185, 311*). For this operation a solution of silver sulfate in fairly concentrated sulfuric acid is said to perform satisfactorily (*89*). A highly recommended adsorbent for ethylene (as well as for the higher olefins) consists of a solution of mercuric sulfate in dilute sulfuric acid. This solution allows rapid irreversible adsorption of substantial volumes of olefins, and its use is not accompanied by any appreciable attack on the paraffin hydrocarbons (*106*). However, this reagent may cause some oxidation of carbon monoxide to the dioxide, and the experimental procedure should be designed to avoid errors arising from this side reaction (*40*). The formulation of mercuric salt reagents especially intended for use in the Blacet-Leighton analyzer has been considered (*241*). A solution of mercuric acetate in ethylene glycol allowed a sharp separation of ethylene from ethane, though it was less satisfactory for the separation of the higher olefins and paraffins. The latter aim was achieved with an aqueous solution of mercuric acetate and nitrate. Results accurate to a few tenths of 1% were secured over a wide range of olefin concentrations. A solution of mercuric sulfate and sodium dichromate in dilute sulfuric acid is said to be very satisfactory for the volumetric determination of total olefins (*185*). A distinction of ethylene from the higher olefins is usually possible, as the former is the only member of the series unadsorbed by 87% sulfuric acid.

A titrimetric method for olefin analysis may be grounded on a measurement of the bromine consumption of the gas specimen (*294*, *298*, *322*). The use of bromine water as an olefin adsorbent in gas volumetric analysis is complicated by the volatility and corrosiveness of bromine. This difficulty is overcome in a compound pipet in which the gas is automatically scrubbed with alkali as it leaves the bromine water chamber (*326*).

The proportion of isobutene in butene mixtures may be established by measurement of the distribution of the sample between 68% sulfuric acid and the residual gas phase (*9*). Isobutene in binary mixtures with other saturated and unsaturated hydrocarbons may be determined with an accuracy of ca. 1% by a simple Bunte buret technique, using as a reagent a dilute solution of hydrochloric acid in saturated brine (*140*). Some correction must be made for the solubility of the second component in this reagent.

1,3-Butadiene. The use of molten maleic anhydride as a specific adsorbent in the volumetric determination of conjugated dienes has been widely investigated, but there are still residual uncertainties in the results of this method. Presaturation of the reagent and a special design for the adsorption pipet have been discussed (*93*, *125*). A careful examination of this analysis, in the form in which a purging stream of carbon dioxide is used to strip the reagent of the gases which, unlike the butadiene, are simply held in physical solution, has revealed several possible sources of error. However, even when these are remedied, the results still show a substantial and apparently capricious variability (*282*). The maleic anhydride method still remains the most satisfactory of the purely chemical procedures for this analysis (*300*); and it may also be of particular value as a method for the concentration of the impurities in butadiene as a preliminary to mass spectrometric evaluation of these contaminants.

The maleic anhydride method is most useful when applied to butadiene samples containing relatively low percentages of hydrocarbon impurities. Interference due to cosorption of these impurities may be reduced, though not totally suppressed, by the addition to the maleic anhydride of various amines, which inhibit the nonspecific adsorption. If large concentrations of isobutylene, one of the most troublesome impurities, are present, it may be preferable to remove the greater portion of this component with 65% sulfuric acid before the maleic anhydride treatment is attempted (*253*).

A titrimetric rather than a gas volumetric procedure may bypass some of the difficulties of the usual maleic anhydride method. If chloromaleic anhydride is used as an adsorbent, only the chlorine atom in the adduct formed through diene adsorption is so activated that it may be determined argentometrically (*240*).

An indirect volumetric procedure for diene analysis is also possible. The total number of double bonds may be established by catalytic hydrogenation, and the total number of olefin and diolefin molecules in the gas may be determined by adsorptiometric olefin analysis. The difference of the former and the latter figures represents the number of molecules with two double bonds—i.e. the dienes (*68*, *249*).

Acetylene(s). Various adsorbent agents for the volumetric estimation of substantial percentages of acetylene have been examined (*310*) and the general problem of acetylene analysis has been reviewed (*307*).

A few hundredths of 1% of acetylene in air may be estimated by adsorption of the acetylene in a cuprous reagent, followed by colorimetric estimation of the cuprous acetylide formed (*65*). For the detection of much smaller percentages of acetylene, a preliminary concentration of this component from a large volume of specimen is requisite. Such concentration may be achieved by passing the test gas through condensation traps cooled with liquid oxygen (*181*) or liquid air (*113*), through an adsorbent tube packed with silica gel and immersed in liquid air (*335*), or through a series of scrubbers charged with acetone and immersed in a dry ice bath (*239*). In the latter case copper acetylide can be formed, and estimated by photoelectric colorimetry, in an aliquot of the acetone solution. When the acetylene has been concentrated in a packed or unpacked condensation trap it may be recovered by warming and flushing the trap. The gas so obtained is brought in contact with a cuprous reagent, and the determination may then be concluded colorimetrically (*113*, *181*). Alternatively, when substantial quantities of acetylene have been collected, the determination may be concluded gravimetrically, by igniting the copper acetylide with nitric acid and weighing as copper oxide (*335*); or volumetrically, by decomposing the copper acetylide with nitric acid and making an iodometric evaluation of the copper that it contains (*339*).

When the acetylene present in a gas stream is collected in a scrubber charged with concentrated aqueous silver nitrate solution, no precipitate is formed until the solution is greatly diluted, at which point a quantitative recovery of the acetylide is secured. The determination is concluded gravimetrically. The gravimetric factor is very favorable and, according to whether a large or small gas sample is taken, the method is serviceable for both minor and major concentrations of acetylene. Interferences due to other gases can be avoided or suppressed, and the method provides a distinction between acetylenes and the alkyl acetylenes, because the latter do not yield precipitates under the experimental conditions (*273*).

Mixtures of acetylene and monovinyl acetylene can be analyzed by argentometric titration (*205*). The separate determination of ethyl- and vinylacetylene in C_4 hydrocarbon gases which may also contain paraffin, olefin, and diolefin constituents can be achieved by a very indirect procedure. If separate measurements are made of the total acetylenes (titrimetrically), total unsaturation (catalytic hydrogenation), olefins (bromination), and butadiene (maleic anhydride), sufficient data for the estimation of the individual acetylenes are available (*322*).

OTHER GASES

Hydrogen. Traces of hydrogen (in the presence of excess oxygen) are determinable from the temperature rise produced when the gas mixture is passed over a catalyst—for example (*59*). Other combustibles should be absent, or the conditions must be arranged so that only hydrogen burns. Hydrogen is particularly easily determined by thermal conductivity methods—for example (*344*).

Substantial concentrations of hydrogen in air may be measured by combustion analysis in a modified Bunte buret (*292*). In an analyzer for hydrogen in flowing gas streams, based on the selective permeability of a hot palladium membrane, the equilibrium pressure of hydrogen in a chamber separated from the gas stream by the membrane is measured (*341*). This pressure should be equivalent to the partial pressure of hydrogen in the gas stream.

Water. A brief review of the methods of determining the water content of gases is available (*202*). Such a determination, based upon an electroconductometric measurement of the equilibrium aqueous concentration of a thin film of phosphoric acid exposed to the test gas, has been very completely studied (*342*). A wide gamut of water concentrations—from a few micrograms to 20 mg. of water per liter—can be evaluated rapidly in a flowing gas system. The calibration of the instrument is somewhat unstable, but satisfactory results are obtained when the time interval between calibration and use is minimized. This analysis is highly specific, but may be extended—for example, to the estimation of traces of combustibles which yield water when they are burned. In an analogous device for the determination of water in flue gases containing sulfur trioxide, a film of sulfuric acid replaces the phosphoric acid (*100*).

For the estimation of a few thousandths of 1% of water in gaseous hydrocarbons the water is adsorbed by cold anhydrous acetone, and this solution is allowed to react with acetyl chloride in toluene, with the liberation of hydrogen chloride. The undecomposed acetyl chloride is then hydrolyzed with ethanol, and the determination is concluded by a titrimetric determination of the hydrogen chloride so produced (*172*). Gravimetric determinations of the water content of Freon 12 (*226*) and of nitrogen dioxide (*347*) have been described. Rapid automatic analysis for water vapor in air may be conducted by thermal conductivity methods. There is a "blind spot" covering a small range of concentrations around the conductivity maximum in this system, but for other concentrations results of high sensitivity and accuracy are obtained (*54*).

Phosphine. Small amounts of phosphine associated with carbide-generated acetylene may be collected in a sodium hypochlorite scrubber. Colorimetric estimation of the phosphate content of the solution, after stoichiometric conversion and exchange to phosphomolybdate and molybdenum blue, is both delicate and fairly accurate (*36, 227*).

Inert Gases. A comprehensive review of the determination of these gases in the atmosphere and in natural gas is available (*51*). Field equipment for rapid determination of the helium content of natural gases has been described (*108*). Helium contents from a few per cent to a few thousandths of 1% are determined by a manometric measurement after all other gases have been removed. The helium and neon contents of the atmosphere have been examined by Glückauf (*116, 117*); the final measurements were made with a Pirani gage after the requisite separations were completed by fractional adsorption. The argon content of various minerals has been determined (*133*), and the analysis of argon-nitrogen mixtures by thermal conductivity methods has been described (*44*).

Ether Vapor. In a gas volumetric procedure for the determination of ethyl ether vapor in the presence of ethylene, etc., hydroferrocyanic acid in dilute sulfuric acid is used as a selective adsorbent for the ether vapor (*189*).

Mercury Vapor. The determination of the concentration of mercury vapor in the atmosphere does not regularly fall within the province of the gas analyst, but the results are of peculiar interest to him. A review of methods for this determination is available (*145*). A very simple procedure in which the test gas is scrubbed in a bead trap charged with alkaline hypobromite solution is concluded by a colorimetric determination of the collected mercury (*198*). A report on the concentration of mercury vapor in gas analytical (and other) laboratories calls attention to the most serious sources of such contamination (*280*).

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CHARACTERIZATION OF ORGANIC COMPOUNDS

ROBERT L. PECK
Merck and Co., Inc., Rahway, N. J.

THE characterization of organic compounds is concerned with a number of fields of experimental study. Establishment of purity is the primary aspect of this problem. Determination of the physical and chemical properties provides a fundamental basis for recognition and identification of the pure compounds. Detailed study of the organic chemistry of a given compound, aided by the results of physical examination, ultimately leads to elucidation of structure, stereochemical configuration, and complete characterization.

It is possible at the present time to characterize new products with confidence and with relative rapidity. This is true of complex synthetic organic compounds, but applies as well to natural products, especially those which are difficult or impossible to obtain in crystalline form.

Numerous vitamins, hormones, antibiotics, and other naturally occurring compounds have been isolated, characterized, synthesized, and put to practical application with surprising efficiency in the past few years. The characterization of these natural products is a result of the practical adaptation of physical methods and the employment of chemical reactions on a microgram scale. The increasing availability of equipment suitable for the precise determination of physical properties of small quantities of material is further reason for present efficiency in characterization work.

This review is concerned primarily with certain recent contributions which have greatly facilitated characterization work in general. It has been necessary to restrict its scope to those techniques and reactions which are most useful for examination of new compounds at the present time.

The primary problem facing the chemist who deals with very small amounts of a new substance of natural origin is the establishment, for reference purposes, of the purity of the material. Many compounds are known which cannot be isolated free of solvent or recrystallized without undergoing significant chemical change. For such compounds, and for others, available amounts of which are too small for extensive recrystallization or fractional distillation, criteria of purity are required before characterizing properties may be assigned with confidence. Methods now available for establishing purity of such compounds are described in the section on purity.

Once purity is established, the physical properties of a pure sample of the new compound are determined. A melting or boiling point determination is made, if practicable. Physical examination, in addition to the melting or boiling point, may now include measurement of ultraviolet and infrared absorption, x-ray studies, potentiometric titration, and reduction or oxidation at the dropping mercury electrode. The most useful physical methods now available are discussed in the section on physical properties.

With completion of physical examination of pure organic compounds, chemical reactions provide the primary information of structural significance which, aided by structural deductions from physical studies, permits the accomplishment of partial or complete structural elucidation. Reactions of most general application are treated in the section on chemical reactions.

PURITY

The classical procedures for preparing organic compounds in a pure state have long been crystallization to constant melting point for solids and distillation to constant boiling point for liquids. It is generally accepted (*4, 13, 23, 92*) that a satisfactory test of purity should include a test of identity and an attempt at separation (*14*). The procedures mentioned above satisfy this requirement, but in many instances cannot be used either because sufficient material is not available, or because the compounds under investigation are unstable when subjected to the conditions of the method. It has become necessary to deal often with very small amounts of material, frequently amorphous in nature, which are nevertheless of such importance that establishment of purity is essential.

Three methods for determination of purity have been developed for application to small amounts of material: the solubility analysis method (*34, 72, 98*), chromatography (*13, 87*), and countercurrent distribution (*22–24*). Utilization of one or another of these procedures has facilitated successful establishment of purity for characterization purposes in many otherwise impossible investigations.

Phase-Solubility Analysis. The application of the phase rule of Gibbs (*32*) to a simple procedure (*34, 72, 98*) has made possible an accurate determination of purity of samples on a milligram basis. The method comprises mixing various quantities of the sample with known volumes of solvent until equilibrium is attained, separation of solid phase from the equilibrium solutions, determination of the concentration of material dissolved in the various solutions, and plotting concentration of dissolved material against total material employed per unit volume. The method is ordinarily capable of detecting the presence of as little as 1% or less of an impurity in a compound. Where one or two impurities are present, it is often possible to determine accurately the amounts of these compounds in the sample.

The measurement of vapor pressures of two or more solutions of a given sample, one containing a slight excess of solid and one containing a large excess, provides a convenient modification of the gravimetric solubility analysis method (*37*). This modified procedure permits more rapid measurement of purity of relatively low molecular weight substances with satisfactory precision, amounts of impurities of the order of 1% or less being readily detected.

Solubility measurements (*95*) can provide strong evidence for identification purposes as well as for homogeneity. The modified gravimetric method cited above (*37*) enables the comparison of mixtures of unknown sample and authentic material to be made easily. The results are as significant as those of the classical mixed melting point determination. It has been shown by this method that admixture of 0.2% of D-glutamic acid hydrochloride with the L-enantiomorph gives a detectable change in vapor pressure; the results indicate that a test of optical purity might be based on this procedure.

Countercurrent Distribution. An apparatus and a process for conducting multiple quantitative extractions in sequence, with small quantities of materials and in a relatively short space of time, have been developed by Craig (*24*). This technique may be applied to microgram and milligram quantities of material with excellent precision. Because it is possible to carry out exact mathematical computation of the distribution of a substance possessing the partition coefficient shown by the test substance, the results may be evaluated with confidence. Details of the process and apparatus have been adequately described in recent reviews (*22, 23*).

This method possesses inherent advantages in that it permits the estimation of composition with precision, provides a precise measure of the partition coefficient of the substance in question, and gives an accurate estimate of the amount and partition coefficients of impurities if these be present.

Chromatography. The use of chromatographic techniques for separation of many classes of organic compounds is now well known. The application of these techniques to testing purity of organic substances is becoming more frequent (*13*). Chromatography of a given substance will usually provide satisfactory evidence of purity if no separation occurs with adequately varied adsorbents and solvents. At the same time, the behavior of the substance under investigation provides a characteristic reference property for identification purposes. Thus, the rate of migration of a single organic substance through a chromatographic column is a function of the chemical nature of the substance, the nature of the adsorbent, and the specific developing solvent employed. In general, the presence of extraneous dissolved substances affects the position of the desired substance on a column only if these substances are more strongly adsorbed. Less strongly adsorbed components proceed ahead of the desired compound and ordinarily do not interfere.

An organic substance may usually be said to be pure if no separation occurs when it is chromatographed on two or more adsorbents in solvents of very different polarities. Very careful evaluation of all available chromatographic data should be made, however, before reaching a final conclusion as to absolute homogeneity of sample by chromatographic evidence.

Several general chromatographic procedures are available, including the orthodox, columnar chromatogram with sectional or flowing elution (*69, 88, 101, 108*), frontal and displacement analysis (*18*), and partition chromatography on silica gel (*52, 53, 105*), paper (*21, 52, 87*), and starch (*59, 60, 61, 86*). The use of ion exchange resins has provided an extremely useful tool for separations (*49*), and has found some adaptation to qualitative identification by chromatographic techniques (*2*). A modification of the paper chromatographic process applicable to small scale preparative work is the recently introduced chromatopile technique (*58*). This procedure, employing a column composed of hundreds of close-packed filter paper disks, permits excellent separation of resolved samples. Quantities of material up to at least 0.5 gram may be conveniently fractionated in this way, and pure fractions recovered in amount sufficient for adequate characterizing examination.

Each of these procedures has inherent advantages for certain types of work. Application of partition chromatography on starch to the separation of amino acids has given an elegant method which permits simultaneous qualitative identification and quantitative estimation of the individual amino acids (*59, 60, 61, 86*). This procedure supplements and extends the outstanding qualitative methods of paper strip chromatography for identification of amino acids. The adaptation of paper strip chromatography to the qualitative identification of sugars (*10, 27, 73, 74*), purines and pyrimidines (*36, 94*), penicillin (*44*), streptomycin (*103*), vitamin B_{12} (*104*), and other organic compounds illustrates the applicability of the general method to qualitative identification work.

The ready adaptability of paper strip chromatograms of both colored and colorless substances to examination by indicators, chemical reagents including oxidizing, reducing, color-forming, and precipitating agents, employment of ultraviolet absorption, polarography, enzymatic treatment, and microbiological assays makes this method of investigation of organic compounds attractive from the identification standpoint. Moreover, the requirement of sample for qualitative characterization is small, on the order of 5 to 100 micrograms. However, the results obtained by paper strip chromatography should be verified wherever possible by the more orthodox methods of organic chemistry. It is anticipated that cases will be encountered where the paper strip chromatographic method will fail to supply adequate differentiation for satisfactory identification.

In view of extensive reviews on chromatographic procedures (*13, 69, 87, 88, 101, 108*) no further comment is made here.

PHYSICAL MEASUREMENTS

Organic compounds are ordinarily examined for melting or boiling point as a first step in characterization. The introduction of the Kofler micro melting point apparatus (*46*) has enabled accurate melting points to be obtained with quantities of sample in the microgram range, in fact, at levels limited only by the facilities for manipulation of samples. The use of the microscope also permits much closer observation of phenomena occurring during heating of sample, such as transition points, sublimation, color changes, and loss of anisotropicity.

Micro boiling points may now be readily determined (*17*).

Numerous measurements such as specific gravity, index of refraction, optical rotation (*33*) and dispersion, neutral equivalent, iodine number, and similar determinations which have long been generally available and are useful in characterization work need only be mentioned in passing.

Many useful physical procedures have become generally available only in the past few years. Infrared spectrography, x-ray measurements, mass spectrography, polarography, potentiometry, Raman spectroscopy, ultraviolet absorption spectrophotometry, and improved light absorption spectroscopy have been adapted for general application. The results of examination by these procedures provide a very helpful asset for characterization of organic compounds. These are mentioned in more detail in the following sections.

Spectroscopy and Spectrophotometry. Useful identification characteristics of colored organic compounds are provided by their specific absorption of radiant light energy (*54, 99, 100*). Equally valuable are the specific absorption spectra exhibited by organic compounds in ultraviolet radiation (*81, 99, 100*). Although neither of these characteristics is usually sufficient as full identification, they are valuable as supplementary properties.

Ultraviolet studies were of value in defining conditions under which penicillin underwent various structural modifications, and were helpful in characterization of rearrangement and degradation products of the penicillins (*107*). A clue to the presence of a nitrophenyl residue in the molecule of chloramphenicol (chloromycetin) was provided by ultraviolet absorption spectra (*80*). This lead facilitated the organic chemical degradation studies which resulted in early elucidation of the structure of the antibiotic. Characterization of α, β-unsaturated ketone groups by means of the strong absorption band appearing in the region of 230 to 260 μ has provided a tool especially useful in sterol investigations.

Raman spectroscopy is being studied to an increasing extent and appears capable of providing both identifying characteristics and structural information (*9*).

The application of the mass spectrograph to identification of organic compounds, especially isotopic materials, is at present somewhat limited, but ample evidence is available that this type of observation provides a useful tool in cases such as gas analysis and will probably have increasing application within a few years (*35*).

Infrared Absorption Measurements. Infrared absorption spectra frequently provide a more nearly absolute characterizing property of an organic compound (*5*). Moreover, recognizable absorption due to groups such as NH, OH, CH, carboxylic acid, carbonyl, ester carbonyl, and monosubstituted amide carbonyl may readily be distinguished and provide very helpful guides to structure elucidation. Infrared spectra of penicillin and penicillin derivatives (*91*) furnished evidence against the oxazolone formula tentatively assigned to penicillin. The strong organic chemical evidence for the β-lactam structure provided by hydrogenolysis studies (*42*) was supported also by infrared studies of the compounds in question. In the elucidation of structure of chloramphenicol (*80*), the organic chemical structure work was also aided by infrared spectrum studies.

X-Ray Measurements. X-ray diffraction patterns of crystalline organic substances provide a very powerful means of identification (*29, 45*). The determination of unit cell dimensions furnishes a guide to molecular size and shape and may be used as a method for determination of molecular weight. Unit cell measurements provided valuable information in the structure work on penicillin (*19, 25*). The extent to which x-ray studies may be applied to organic chemical structural elucidation is well indicated in the work on penicillin (*25*). Confirmation of molecular weight and indications of flat molecular configuration were given for biotin by x-ray measurements (*28*).

Polarography. The polarograph has been successfully applied to identification and quantitative estimation of a wide variety of redox groups (*67, 97*). With pure compounds possessing an oxidizable or reducible group of known type, polarography yields information on equivalent weight, as well as usually satisfactory identification of the nature of the group.

Potentiometry. The improvement in techniques of potentiometric titrations of milligram to microgram amounts of sample has extended the availability of a most useful procedure (*56*). Potentiometric studies of hydrolytic cleavages provide highly valuable information on the products with respect to compound types and acidic and basic strength, as well as information on rates of hydrolysis. Potentiometric investigation of redox and oxidative reactions also may be carried out on very small scale samples with useful results. Equivalent weight data are often most easily determined by potentiometric measurements. Dissociation constants determined by potentiometry provided valuable information in the characterization of penicillin and its derivatives (*107*).

Molecular Weight Determination. Determination of molecular weight by various means has long been possible. Adaptation of various methods to the milligram and microgram scale in recent years has supplemented other phases of characterization of complex organic substances. The Northrop diffusion method (*71*), the Rast procedure (*79*) and its modifications (*70, 76*), and the Barger isothermic distillation procedure (*3*) and its modifications (*57, 70*) all represent useful techniques which have been adapted to a conveniently small scale. The ebulliometric (*89, 90*) and cryoscopic (*83*) methods adapted to a micro basis provide accurate procedures which are very frequently applicable.

CHEMICAL REACTIONS

Subsequent to Pregl's introduction of techniques for quantitative organic microchemical analysis (*78*), there have been ever-increasing development and extension of methods of elemental analysis and group analysis (*31, 84, 102*). The results of such studies have provided a sound basis for analytical characterization of organic compounds. Accurate, significant data may readily be had on elementary composition, empirical formulas, equivalent and molecular weights, and the equivalent proportions of such groups as hydroxyl, amino, *O*- and *N*-acyl, alkoxyl, alkimino, *C*-methyl, carbonyl, carboxyl, and halogen groups (*17, 43, 78, 82*). Methods for micromanipulation of small-scale organic reactions have generally been developed as the occasion demanded; these techniques have been extensively discussed in recent reviews (*6, 7, 102*).

The presence of a wide variety of characteristic organic functional groups may be detected by application of specific, qualitative tests worked out for microgram quantities using spot test techniques (*30*). Knowledge of the nature of functional groups present in a molecule of incompletely known structure may enable a colorimetric assay to be devised for following the color-producing moiety during degradative studies. The application of the Sakaguchi test to follow changes in the guanido groups and of the Pauly test to show concomitant formation of imidazole groups in degradation of streptomycin and streptidine (*12*) serves as an illustration of this principle.

Knowledge of functional groups also permits introduction of a wide variety of tracer groups—e.g., halogen-containing acyl groups, sulfur-containing residues, isotopic residues, radioactive groups, ultraviolet-absorbing groups, and the like—which are often of utmost importance in recognition of one or another of the fragments of complex or conjugated molecules during the subsequent degradative reactions, and may also be of value in determination of equivalent and molecular weights. More general application of such techniques is possible with the increasing availability of sensitive methods of evaluation.

Microbiological assays provide a means for identification of various organic compounds possessing biological activity (*26*). Supporting evidence is usually necessary for confirmation of identity, but the remarkable specificity of microbiological assays for certain amino acids (*68*), vitamins (*41*), antibiotics (*96*), and various other compounds (*77, 85*) provides a rapid and useful tool for identification of many organic compounds as well as for quantitative estimation when they are shown to be present in a mixture. These methods are frequently helpful when mild degradation of a biologically active molecule is to be carried out.

General procedures for classifying organic compounds by solubilities and functional group reactions (*17, 38, 43, 55, 82*) provide leads for selection of derivatives suitable for further characterization. The more commonly employed and most generally useful derivatives are fully discussed in the references cited. The value of derivatives from which the parent compound may be regenerated by mild conditions such as hydrogenolysis—e.g., carbobenzoxy derivatives (*8, 15, 16*)—is emphasized. Such derivatives are of especial importance when it is necessary to regenerate a derivative of a biologically active substance to recover original active substance. The choice of less commonly used derivatives will usually be determined by considerations of specific need, such as ease of recrystallization or regeneration and availability of known or obtainable reference compounds.

In so far as known and previously characterized organic compounds are concerned, the general chemical methods cited above, together with the physical methods of examination discussed in the preceding sections, usually provide more than adequate means of differentiation from other compounds, of identification, and of recognition of organic structure.

New and previously unknown compounds when examined by these methods will yield physical and chemical properties sufficient for recognition and identification purposes. When, however, it becomes necessary to elucidate the full organic chemical structure and stereoisomeric configuration if this is involved, an extensive and detailed study of the behavior of the new molecule in a wide variety of specific chemical reactions is usually required. Comparison of suitable derivatives or degradation products or both with known reference compounds must be carried out. The selection of reactions will be based on the leads gained from preliminary observations which will have given considerable information on the probable size, type, and empirical formula of the molecule in hand. For example, it is ordinarily possible to determine with reasonable certainty whether hydroxyl, amino, alkoxyl, acyl, carbonyl, or carboxyl groups, unsaturated bonds, aromatic rings, sulfur, phosphorus, or halogens are present. Discussion of reactions found particularly useful for degradative characterization work is limited in this review to a few general types.

The most useful reactions for degradative purposes are obviously those which are applicable to the milligram to microgram scale, which give quantitative or semiquantitative recoveries of product, or which provide quantitative information on the extent of oxidation, hydrogenation, or other desired reaction, and which are conclusive in terms of structural significance.

Hydrolysis, acid or alkaline cleavage of conjugated molecules, is a fundamental reaction from which the fragments may be isolated for further study. Potentiometric examination of the reaction during its course often yields helpful information on basic strength of formed products, dissociation constants, and rate of reaction. The use of physical measurements—e.g., potentiometry, ultraviolet absorption, infrared absorption, optical rotation, and the like—for following cleavage reactions, procedures which are now possible as a result of increased availability of suitable instruments, has augmented the accepted value of hydrolytic reactions. The recent work on elucidation of structure of actadione (*47*) is of interest in this connection. The degradative work on penicillin and its derivatives provides many illustrations of the structural significance of such studies (*20, 107*).

Hydrogenation reactions have become increasingly important in characterizing work, for the yields of product are usually high, the reactions generally go to completion, and the resulting products possess properties favorable for subsequent treatment. The recent developments leading to wider application of hydrogenation procedures justify some discussion of these reactions.

Quantitative hydrogenation in neutral solution with platinum catalyst and hydrogen at atmospheric pressure effects reduction of aliphatic carbon to carbon double bonds, unhindered carbonyl groups, nitro groups, nitriles, and oxygen- or nitrogen-substituted benzene rings (*1*). Unsubstituted and alkylated benzene rings are resistant to these conditions, but are reduced when mineral acid is present in the medium. The quantitative determination of molar equivalents of hydrogen absorbed by an organic compound under controlled conditions provides valuable structural information. Hydrogenation of double bonds usually yields products which are more stable than the parent compound and may be handled more easily.

Sulfur hydrogenolysis reactions with Raney nickel catalyst (*64*) have proved to be of utmost importance in dealing with compounds containing sulfur in the form of sulfhydryl, sulfide, disulfide, thio ester, and thio ether groups (*40, 42, 48, 62, 66*). The quantitative replacement of the sulfur atom by two hydrogen atoms gives a desthio derivative which is usually more stable than the sulfur-containing molecule, and which can be much more readily handled in subsequent reactions. The desulfurization of biotin (*93*) was conclusive in establishing the correct structural formula for that compound. The extensive application of the method during penicillin studies further illustrates the utility of the reaction (*42, 63, 75*). The reaction may also be employed to good advantage when aldehyde groups are to be converted to methyl groups (*106*). Formation of the diethyl mercaptal derivative followed by desulfurization with Raney nickel catalyst yields the desired methyl group. This method was employed in formation of tetraacetylbisdesoxystreptobiosamine (*48*) during studies on streptomycin structure.

The use of Raney nickel catalyst, in sufficiently large quantity relative to sample, effects hydrogenation of carbon to carbon double bonds, carbonyl, azoxy, and hydrazo linkages and the like without the presence of added gaseous hydrogen (*65*). The hydrogen involved is that adsorbed on the catalyst. Nonactivated benzene rings, aliphatic acids, and esters are stable to reduction under these conditions. Aromatic alcohols may thus be converted to the corresponding aromatic hydrocarbons, as may aromatic aldehydes; thus at 78° benzaldehyde gives toluene and not benzyl alcohol, whereas at 25° benzyl alcohol is obtained. The temperatures required for these hydrogenations are reasonably low, ranging from about 25° to 100° C.

Hydrogenation of carbon to carbon double bonds in compounds containing divalent sulfur using hydrogen and palladium on carbon or palladium on barium sulfate has been worked out for use in the generally available low pressure (below 50 pounds) hydrogenation apparatus (*64*).

Among numerous oxidative reactions, which have been employed in degradation of organic compounds, the reaction of periodic acid with glycols (*50, 51*) is of outstanding importance. The reaction is subject to quantitative interpretation, may be used with very small amounts of sample, and yields highly significant structural information. Media which have been employed for this oxidation include acid, neutral or buffered aqueous solutions, and aqueous-organic solvent mixtures. In compounds containing two adjacent hydroxymethylene or primary aminomethylene groups, there occurs oxidative cleavage of the carbon to carbon bond joining them, with formation of two aldehyde groups. If more than two adjacent hydroxymethylene or aminomethylene groups are present in the molecule, the carbon moiety of each of those lying between the end members of the series is oxidized to formic acid, which may be determined quantitatively. Terminal hydroxymethylene groups are converted to formaldehyde by the oxidation, in which case quantitative estimation by dimedone precipitation permits accurate deduction as to the number of such terminal groups in the molecule. An elegant elucidation of structure of streptamine depended primarily upon periodate oxidation reactions (*11*). Application to determination of structure of chloramphenicol (*80*) further illustrates the utility of periodate oxidation. An extensive review adequately covering details of the methods of periodate oxidation and applications to structural work on sugars and other substances has recently appeared (*39*).

It is beyond the scope of the present review to present more than a brief survey of the more significant recent developments in characterization of organic compounds. The wider application of sensitive physical methods has greatly facilitated organic structural work, although fundamental organic chemical reactions still provide the basis for ultimate elucidation of structure. However, the coordination of both chemical and physical observations is necessary for most efficient characterization.

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BIOCHEMICAL ANALYSIS

PAUL L. KIRK and E. L. DUGGAN, *University of California, Berkeley, Calif.*

THE biochemical analyst is confronted with a set of problems quite different from those of chemical analysts. Classical analytical chemistry is concerned primarily with identification and determination of the ultimate units, elements, and radicals of the chemical system; biochemical analysis is directed toward the operating or functional units of the complex biological system. The functional units may be on a cellular, high molecular, or simple molecular level. Thus the carbon content of a protein has little significance as compared with the active groups and functional units of that protein. The fat content of the biological system is far less significant than the activity and structure of lipoprotein or other lipide complexes. In a similar way, the phosphorus content is unimportant except as it indicates active metabolism through the mediation of numerous phosphate compounds, stable and unstable.

Biochemical analysis is inherently a more complex and broad field than classical chemical analysis. Because the major activity of the biochemist is always to find out "what" and "how much," biochemical analysis actually must include virtually every biochemical technique. Even in the study of rate and mechanism, analysis at appropriate intervals and under special conditions is the primary requirement. Almost all of the specialties of the chemical analyst must be considered as tools of the biochemist also. Of necessity, any discussion of the field must overlap greatly the specialized subjects already reviewed in considerable detail in this and other journals. Further complication arises from the fact that a single biochemical problem may call for the application of widely diverse techniques, or at least for a difficult choice between a number of possible techniques.

The preparative stage of most biochemical analyses consists of some type of fractionation or separation. These processes are then a most important part of the field reviewed. The further application of classical procedures for analysis of elements or radicals in the fractions usually follows as a requirement for quantitative evaluation of the fractions. This rather secondary phase has pre-empted much of the attention given biochemistry by the analyst.

Sampling procedures in biochemistry do not aim only at uniformity and therefore total representation of a large system; more often these procedures are concerned with the minutiae of the heterogeneous system. It is more significant to know the variations from tissue to tissue and from part to part than to determine the average composition of the whole. Even more important, any knowledge that can be obtained without alteration of the system is more valuable than information obtained after applying the destructive techniques of ordinary chemical analysis. From the biochemical standpoint, the ultimate aim is to obtain analytical data from completely unaltered biological systems. Because the ultimate unit of life is the cell, the final goal must be an analytical understanding of the component structures and composition of the cell. This dictatorship of the cell determines the units and their relative importance. Because the cell is small and contains many significant components in small amount, specific cytological and microchemical techniques of proved sensitivity are required. The necessary sensitivities have been only partially achieved, and in very limited aspects. For these reasons, development of a biochemical analysis method is one of the most important needs for the elucidation of the mechanisms and nature of life processes.

The considerations treated briefly above are kept in mind in choosing the material reviewed here. The final value of any research publication is considered more on the basis of its probable future value than of its past accomplishments. Therefore, emphasis is placed on those techniques for which a continuing future application can be foreseen, rather than on those which appear to be approaching the limit of their utility.

FRACTIONATION PROCEDURES

Every analysis proceeds through two stages, the preparative and the determinative. The first stage is chosen on the basis of the nature of the sample, the interfering materials present, and to a certain degree, the nature of the determinative step. In biochemical analysis, this preparative step is the most important. It usually consists of a fractionation of one type or another, and may be carried out at the simple molecular level, the high molecular level, or the cellular level.

SIMPLE MOLECULAR LEVEL

Two of the more important fractionation methods at this level are chromatography and extraction. Both may be expected to have an increasing rather than diminishing value in biochemistry.

Chromatography. This highly valuable technique has been reviewed in this journal (*178*, *277*) and elsewhere (*73*, *105*, *206*, *322*). Its most significant use has undoubtedly been in the separation and isolation of biological materials, for which reason reviews have dealt most extensively with its biochemical aspects. It is not the purpose of the present review to present a complete discussion or exhaustive references. Rather, it is hoped to indicate the significance of the development as a part of the working technique of the biochemist.

Numerous variations of adsorption or partition analysis (chromatography) are employed. Adsorption or partition on various materials in columns and on paper are the two techniques almost always utilized. Either may involve ion exchange (*9*), partition between a stationary and moving solvent, or surface forces alone. More commonly, two or more of these effects are present simultaneously, though a distinction is usually made between them in designating the process.

The biochemical significance of adsorption analysis either in columns or on paper rests on the simplicity of the method, its versatility, and its very great sensitivity to minute differences in structure and properties of the materials being separated. This allows isolation of the individual constituents from mixtures of components so similar as to defy most other methods of fractionation. The simultaneous occurrence in biological systems of compounds that differ from each other in very minute degree is so common as to require procedures of this sensitivity for separations and identifications. The first well-known use of chromatography by Tswett in 1906 was in separating plant pigments even yet not readily fractionated by other methods.

The greatest deficiencies of the method from the biochemical standpoint are the necessity of identifying by auxiliary means the constituent or mixture comprising each band or spot, the occasional difficulty of locating the various constituents when no simple tests are available for a colorless and nonfluorescent material, and the difficulty of applying the method in a strictly quantitative manner.

The widest application of the chromatographic techniques has been in the separation of amino acid mixtures, either by column methods such as that based on the use of starch by Stein and Moore (*272*), or the paper methods based largely on early work by Martin and Synge (*208*) and Consden, Gordon, and Martin (*74*). The starch column method (*98*) is capable of yielding accurate quantitative analyses of amino acids in mixtures (*272*). It has also been applied to the quantitative analysis of ribonucleosides by Reichard (*249*), to purines by Edman *et al.* (*99*), and to extensive and accurate analysis of purines and pyrimidines by Daly and Mirsky (*86*). It is a rather complex procedure as compared with the paper methods. It requires much personal attention to collect small samples at regular intervals, or the comparatively expensive substitution of a mechanical device for this collection (*272*). The method has been avoided by many investigators for these reasons, though it is clear that starch columns are superior to paper in several respects. Numerous other adsorbents have been and continue to be used in columns, depending on the materials separated and the experience of the investigator. These include a wide variety of adsorbents and ion exchange materials (*26, 285, 311, 312*). The possibilities in separating small quantities of unusual materialsi are llustrated by the work of Wald and Allen (*307*), who differentiated the eye pigments of Drosophila on talc.

Paper chromatography is inherently a simple process, calling for little special equipment and few unusual reagents. As applied to amino acids, it is capable of producing delicate separations, partially by virtue of its ability to separate in two dimensions. Preliminary separation into groups is achieved by passing the solvent along the paper in one direction. The solvent is substituted or altered and passed at right angles to the first direction, giving further separation in the second direction. This leaves the amino acids as a series of spots distributed in a largely predictable pattern. The spots are located ordinarily by application of ninhydrin and heating. They are identified by comparison with known amino acids treated identically, and an approximation of their amount can be made by observing the length (*109*), the area (*28, 34, 109*), or the intensity (*28, 34, 48, 109, 114*) of the spots. Various efforts have been made, with partial success, to determine the amounts of the various amino acids accurately by photometric evaluation of the ninhydrin color after elution (*12, 180, 223*). Impurities in the paper and in the reagents, combined with difficulties of standardization of the conditions of color development, have usually prevented dependable results from being obtained (*207*). The employment of the Kjeldahl method for total nitrogen (*207*) and a variety of methods for determination of amino nitrogen (*207*) have met with partial or total failure from the same general causes.

Perhaps the most successful analyses reported to date (*207, 316*) are those using the rather specific but not highly sensitive copper method similar to that of Pope and Stevens (*242*). Radioactive carbon or sulfur incorporated in the amino acids has also allowed the estimation of amino acids in the spots with reasonable success (*25, 108, 169*). Similar use of tracer sulfur was made by Tomarelli and Florey (*300*) in studying urinary sulfur compounds after separation on paper. Tracer iodine was similarly used by Taurog, Tong, and Chaikoff (*290*) to evaluate the fractions of thyroid iodine. Many times, radioactive tracer technique will not be applicable because of the difficulty of incorporating enough tracer in the numerous compounds of the living organism.

Paper chromatography has been extended to the separation of many compounds of biological interest other than the amino acids. Some recent applications include separation and estimation of peptides (*36, 162, 285*), nucleic acid derivatives (*54, 62, 64, 133, 153, 204, 305, 306*), sugars and methylated sugars (*23, 44, 111, 113, 121, 155, 159, 228*), organic and fatty acids (*199, 205, 232, 246*), keto acids (*60*), alcohols (*310*), aldehydes (*190*), amines (*101, 286*), enzymes (*62*), creatine and creatinine (*6, 210*), ergothionine (*320*), steroids (*321*), vitamins (*315*), penicillin (*167*), and other compounds (*63*). Steroids have also been separated on adsorption columns (*10, 142, 301*), while ion exchange resins have been employed for separation of basic amino acids (*141*), nucleic acid derivatives (*54, 71, 76, 103, 269*), and riboflavin (*118*). An interesting application of the adsorption column was made by Riley *et al.* (*251*), who separated subcellular enzymically active granules on Celite.

Several recent extensions and improvements of technique in the application of paper chromatography have appeared (*94, 135, 200, 213, 220, 252, 255, 275, 303, 313, 317*), as well as extensions of theory (*42, 204*). It is curious that filter paper, a universal item of the chemist's equipment, should be found to be the best material for two-dimensional chromatographic purposes. To the extent that only partition is involved, this is not surprising, because then the purpose of the paper is a passive one—i.e., to immobilize one liquid phase. If other factors are present—e.g., adsorption—then paper may fall far short of other materials. Certainly, the impurities of paper have led to great difficulties in the quantitative evaluation of the separated materials. Filter paper is not pure cellulose, even when it is uncontaminated by laboratory fumes and dust. Some attempts have been made to replace it in columns and on slides (*211*) with powdered cellulose (*155, 231*), cellulose acetate, and other materials. As with other laboratory reagents, the material used for chromatography should be purified to the necessary extent, and adjusted to its optimum condition for the purpose at hand. This development may be expected.

Paper chromatrography, particularly, is capable of separating and isolating very minute quantities of material (*2*) and is therefore of special interest in the biological microsystem. It has, in fact, outrun the development of microchemical methods for analysis of the separated quantities, and has not often been fully utilized at its limit of sensitivity. In general, the amounts separated are in the microgram range, and may often involve only fractions of a microgram. The extensions to compounds and biological systems other than those mentioned are unlimited in their possibilities. It can be safely stated that chromatography will be responsible for many important accomplishments in the future of biochemical analysis.

Extraction. As a means of fractionation at the molecular level, prior to the determinative phase of quantitative analysis, extraction has long been a standard technique for some biological constituents such as fats and sterols. Until recent years, the applications of extraction have been unimaginative. Even yet, little advantage has been taken of the many possibilities of this technique in routine analysis. During the past ten years, a number of new and selective solvents have been developed commercially, but most of these have as yet no biochemical use because of the lack of exploitation. This is regrettable in view of the tremendous developments in solvent extraction techniques in research and in industry, during and immediately after the war. The simplicity and cleanliness of the extraction methods commend them for use in fractionating any kind of complex system. The conditions for obtaining clean-cut separations are not always easy to determine, and in fact, the solubility relations of the pure material are not always sufficient to predict its behavior in complex mixtures (*97, 146*). A few widely scattered efforts have been made to extend the application of more efficient and selective solvents in separation of organic acids (*131, 227*), amino acids (*3*), drugs (*89*), and a few other materials. It seems that the criterion of importance must certainly apply to this relatively neglected field.

A more productive trend in extraction method resulted from the development by Craig (*81*) of an apparatus for rapid application of what he terms "countercurrent distribution." This subject has been recently reviewed by Craig (*79*). The procedure, which is highly efficient in separating materials having solubilities or distributions very close to each other, consists of means whereby a series of multiple extractions is carried out in sequence in the same apparatus. Equilibrium is reached in each set of extractions, thus allowing an exact mathematical treatment. The method has given results with problems that had resisted all other methods of attack. The technique is more easily put into operation than is likely to be true of chromatography, for example. The difference resides largely in the fact that a solvent giving a suitable distribution can usually be found with little difficulty and applied immediately. Chromatography may require considerable empirical testing before a suitable combination of adsorbent, solvent, and developing agent can be found.

The Craig countercurrent distribution apparatus and technique have been employed qualitatively and to a lesser extent quantitatively in the study of purines and pyrimidines (*295*), diphosphopyridine nucleotide (*150*), protein hydrolyzates (*20, 319*), fatty acids (*11, 257*), drugs and antibiotics (*21, 35, 80, 82, 298*), preparation of oxytocic material (*197*), and a variety of organic compounds of limited biochemical interest. A particularly interesting application was that of Bowden and Peterson (*37*), who were able to separate biotin bound to protein after hydrolysis with acid or an enzyme. It is safe to predict that this technique will be adopted progressively more widely as its advantages become generally realized. It will serve rather as a supplement to chromatography than as a competitor. Many times its use will be found simpler and equally decisive.

HIGH MOLECULAR LEVEL

In the biological system there exist many compounds and complexes of very high molecular weight. Most plentiful are the proteins, while nucleic acid, starch, and glycogen are important. The structure and properties of protoplasm appear to depend on labile and incompletely understood combinations of these high molecular weight materials. Much research will be necessary to subject all of the high molecular compounds to analysis and suitable fractionations, but considerable progress in this direction has been made.

Fractionation of Proteins. ALCOHOL FRACTIONATION. A frequent fractionation required by biochemists is the separation of a particular protein from a mixture of other proteins and smaller molecules. The classical procedures make use of isoelectric precipitation or, where that is impossible, of salting-out procedures using ammonium sulfate or other soluble neutral salts. Wherever salting-out is employed long dialysis is required to remove the salt from the desired protein fraction. Such dialysis procedures slow the preparation of a desired protein with consequent danger of bacterial contamination or of denaturation.

During the war the research teams concerned with the preparation of human plasma albumin were able to develop an ethanol fractionation system capable of great utility in biochemical preparations. The advantage of the procedure is the use of a solvent during fractionation which may be completely and rapidly removed by vacuum drying at low temperature.

The early techniques evolved by the Harvard group and their collaborating laboratories were fashioned to yield pure human albumin and several by-products, including fibrin in various forms and a mixture of globulins (*40, 70, 100, 221*). The various methods utilize controlled variations of ionic strength, ethanol content, pH, and temperature. The temperature is kept close to the freezing point of the mixture at all times, and thus may be progressively decreased with increase in ethanol content. The tendency of alcohol to denature the proteins is thus kept at a minimum. Continued research has resulted in further fractionation of the plasma globulins to yield proteins of enzymatic, antibody, and metal-combining activity (*87, 88, 281*). Crystalline human and bovine albumins have been obtained from ethanol-prepared concentrates (*69*).

Since the development of the ethanol fractionation system, a similar technique has been used to examine serum protein changes following injury (*125*). The system has also been successfully applied to the preparation of purified bacteriophage of the T_6 variety (*177, 245*). An analogous fractionation, using methanol rather than ethanol, has yielded a simple method for preparation of hemagglutinins for blood typing (*235*), crystalline tetanus toxin (*234, 238*), and diphtherial toxoid (*236, 237*).

While the fractionation of plasma has already been of great practical importance, the fractionation system developed should be of great continuing importance in the preparation of numerous proteins. The method, with proper precautions to eliminate local heat of mixing and local overconcentration of alcohol, should be generally applicable to protein mixtures. Tests of the method are obtained in fractions where the desired product has measurable activity. Thus, the preparation of a highly active or crystalline enzyme by means of the system enables the essay of activity as purification proceeds. The demonstration of the yield of enzyme at any step in the procedure will define the conditions which prevent enzyme recovery by inactivation, unexpected solubility, or precipitation with other proteins. Rapid development of alcohol fractionation procedures may be expected in the future as such tests are applied to various steps of the treatment. Advantages of the alcohol procedure from a commercial viewpoint are the low cost of the alcohol and its recovery during the final vacuum drying step. Disadvantages are the initial cost of the refrigeration equipment and lyophilizers necessary.

PREPARATIVE ELECTROPHORESIS. The use of electrophoretic migration as a means of estimation of the homogeneity and mobility of proteins is well known (*198, 297*). This phase of electrophoresis is one of the determinative techniques of biochemical analysis.

As a preparative procedure, electrophoresis has been used to separate amino acid mixtures into basic, neutral, and acidic fractions (*78, 115*). Similarly, proteins possessing different isoelectric points may be separated by migration at a pH between their isoelectric points (*158, 296*). Consden *et al.* (*75*) applied electrophoretic transport in a silica gel medium to separate acidic, neutral, and basic amino acids or peptides from hydrolyzates. Their method yielded satisfactory separation for minute amounts of hydrolyzate. The determination of composition and arrangement of amino acids in the antibiotic, Gramicidin S, was possible by the new technique coupled with effective use of paper chromatography.

Proteins possessing similar isoelectric points but different net charge may be separated in the Tiselius apparatus (*132*, *292*) by virtue of different rates of migration. Such separations involve techniques suitable for small amounts of dilute protein solutions. However, the recent preparative apparatus designed by Svensson (*284*) allows the use of electrophoretic separation on a "semicontinuous" scale.

The phenomenon of electrostratification (*222*, *284*) has been applied to the problem of separating very similar proteins by Kirkwood and his co-workers (*52*, *53*, *173*, *226*). The apparatus is designed in such a way that horizontal electrophoretic transport and vertical convective transport of a protein fraction occur simultaneously. Thus, the mobile fraction migrates toward one electrode, a density gradient results, and the mobile component accumulates by convection in the lower reservoir of the apparatus.

CELLULAR LEVEL

In the elucidation of structure and composition of living matter, it is not sufficient to rely on chemical separation alone. The smaller units of tissues—viz., the cells and their internal structures—are far too small to segregate individually in sufficient quantity for present analytical procedures. Yet, if knowledge of these cellular structures is to be obtained, they must be isolated in relatively pure form. Their fractionation is no less significant to the biochemical analyst than the chemical fractionations used for molecular species.

Differential Centrifugation. The use of a centrifugal field to fractionate a biological system before analysis is an old technique, which has evolved into one of the most fruitful methods by which the various cell structures may be isolated and characterized. A necessary adjunct of the various high speed centrifuges for biochemical work is a refrigeration unit, in view of the labile nature of the enzymes and proteins present. Thus, heating of the solutions during treatment is minimized and greater speeds are possible. Further progress in this field should follow the recent appearance of centrifuges of the type of the refrigerated preparative ultracentrifuge.

The separation of plant or animal cells into nuclei and cytoplasm, followed by further fractionation on a centrifugal basis, before chemical treatments are applied, would seem to be one of the most promising procedures for the biochemical analysis of cells. Before chemical treatment is employed, other than procedures demonstrated to be innocuous for the fraction desired, a certain amount of histological control may be used.

The chief components of the nucleus are the chromosomes which exercise hereditary control. Other functional components are unknown. The nuclei may be obtained following fragmentation of cells by grinding, blending in a high speed mixer, or passing through a colloid mill. After fragmentation, nuclei may be found in the residue from centrifugation at low speed. The problem of preparation of nuclei and cytoplasmic particle fractions is chiefly one of obtaining one fraction uncontaminated with another. Thus any advance in the mode of preparation of mitochondria (large cytoplasmic particles) is a similar advance in the preparation of purer fractions of nuclei. Advances have been made in the preparation of nuclear fractions for enzyme studies (*149*, *179*, *261*) and analysis (*92*, *93*, *224*, *261*).

Great interest exists in the composition and structure of the chromosomes of cell nuclei as a consequence of their relation to genetic behavior. Although the work in separating and identifying components of the chromosomes is extremely difficult, fractions high in chromosome content have been obtained (*68*, *214*–*216*) and estimations of histone, residual protein, and the two types of nucleic acid have been made (*215*, *217*, *218*).

The mitochondria (particles 0.5 to 2 microns in diameter) were the first cytoplasmic fraction obtained from cells (*24*, *148*). More recently the smaller particles, microsomes, have also been separated (*66*, *67*). These are considered to be lipoprotein. Similar small particles composed mainly of glycogen have been isolated (*181*, *182*). While the early separations of particles from cytoplasm were made with isotonic sodium chloride as extraction agent, the use of 0.3 to 0.9 M sucrose solutions has proved to be a great improvement in technique (*152*). Previously, the saline extract yielded clumped nuclei and large particles in one fraction, whereas the present method enables more efficient separation of several possible fractions. A junction between histology and biochemical analysis was accomplished during the isolation of mitochondria, inasmuch as the typical staining properties of these particles were used as a control in the work.

The present efforts to investigate the various particulate fractions of rat liver cytoplasm are aimed toward comparative analysis of the fractions (*19*, *152*, *168*, *188*, *244*, *262*) and the exposition of the enzyme systems which the fractions contain (*151*, *168*, *185*, *188*, *219*, *261*, *262*). The mitochondria have been shown to contain a great proportion of the pentose nucleic acid of cytoplasm as well as protein and phospholipide. Certain oxidative enzymes have already been shown to be present in these large particles. More rapid progress may be expected in this field.

The fact that certain enzymes are shown to be associated with the large granules and not with other fractions would seem primary evidence of the successful fractionation of the cells. This follows from the tremendous activity of unweighable traces of enzymes. Such tests are especially useful because they provide reasonable certainty that the fractions have remained native—i.e., they have not been inactivated by the procedure used. The further determination of chemical composition of such tested fractions would thus be likely to represent conditions in the complete cell. It would seem necessary that the advances in technique of cellular fractionation should be considered before chemical fractionations of tissue are attempted. For example, it would appear logical to remove nuclei as such before beginning the preparation of pentose nucleic acid by chemical means, inasmuch as this nucleic acid is chiefly concentrated in cytoplasm. Thus a histological fractionation may precede the biochemical fractionation in many investigations.

This portion of the review has concerned itself chiefly with the centrifugal methods as applied to cytochemical structures. A vast field of development in the use of these methods exists in the preparation and purification of the viruses. The viruses, while having a variety of sizes and biological effects (*176*), are considered to be nucleoproteins. Because their molecular weights are generally greater than a million, they are most easily separated in active form by differential ultracentrifugation. As in the case with the enzymes, the biological activity of the viruses may be used as a control for the determination of the best conditions of preparation.

Although not properly a part of differential centrifugation, the use of the ultracentrifuge in the determination of molecular weights of proteins and other large biological molecules is of paramount importance. The sedimentation constants or molecular weights are a part of the quantitative evaluation of such functional units. The classical ultracentrifuges and techniques were adequately reviewed (*233*, *260*, *283*). The chief advances since the last review have been refinements of techniques and application of the method to diverse materials. The present availability of commercial electrically driven ultracentrifuges will result in widespread application of centrifugal analysis to the determination of molecular weights of proteins under various experimental conditions.

DETERMINATIVE PROCEDURES

The great advances in fractionation procedures have allowed the final determination of most constituents to be carried out by simple methods applied to the fractions. Determinative methods also have advanced steadily and impressively. In particular, the use of photoelectric photometers and spectrophotometers in the visible range has been broadened and intensified greatly. This field is considered to be far too vast and well understood to justify discussion in this review.

Another important approach is the application of methods which are actually determinative in nature, but may be applied to the unfractionated system to obtain knowledge of its composition, structure, and arrangement. In this category, direct absorptiometry is most significant. Certain uses of isotopes are also included, as are some of the respirometric procedures. All these general techniques are capable of application likewise to the fractionated or specially prepared system.

ABSORPTIOMETRY

Absorption of radiation by solids or dissolved materials is one of the most versatile tools available to the biochemist. The ordinary color development and measurement fall in the realm of visible light absorptiometry. Such color development follows chemical alterations more or less destructive to the original biological system. This is far less true of absorption measurements of infrared and ultraviolet radiations, x-rays, and Raman spectrometry, all of which are less widely recognized and employed. These methods can often avoid the destructive preparative steps of analysis. Although not new, these techniques are in their early stages in the biochemical field, and will certainly increase in importance with time. Their value lies in the fact that the type of absorption curve obtained is somewhat characteristic of the compound or its groups and bonds, and may therefore be used for quantitative and qualitative purposes. It is particularly valuable for elucidating structure, complex formations, and related molecular alterations. This is entirely analogous to the specificity of measurement of colored compounds using visible light absorption.

Ultraviolet Absorption. The measurement of ultraviolet light absorption is relatively much further developed than other phases of nonvisible radiation absorptiometry. A chief reason is clearly the easy availability of good and relatively inexpensive instruments for this purpose, rather than any necessary superiority of ultraviolet light over other invisible radiation. Another reason is that aqueous solutions are amenable to study. In general, ultraviolet wave lengths are absorbed by conjugated double bonds, aromatic and certain related ring structures, a variety of inorganic ions, and some other materials. Biologically important compounds containing such structures may then often be detected and estimated by the amount of the absorption at some specified wave length. The general field of ultraviolet absorptiometry has been reviewed recently (*253*).

Perhaps the most significant development of ultraviolet absorptiometry in biochemistry has been its extensive application to the study of nucleic acids, both in the intact cell and quantitatively in isolated samples. The absorption of these compounds depends on their contents of the purine and pyrimidine ring structures. Because of the tremendous significance of nucleoproteins in biochemistry and genetics, the availability of this tool has stimulated greatly the amount and quality of the research and its results. The absorption by nucleic acids and their derivatives in the ultraviolet has been extensively studied (*46, 59, 104, 163, 240, 241, 276, 278*). Its use in direct analysis is now too widespread to note individual references. In general, the wave length used for analysis of total nucleic acid content is in the neighborhood of 2600 A., though this may be varied when certain fractions are being determined. A very interesting application of ultraviolet absorption was the work of Caspersson (*56*), who made extensive use of the quartz microscope and microabsorption measurements for the study of cell structures containing nucleic acids and to a lesser extent other absorbing cell constituents such as the proteins. The result of this work has been a series of highly important research publications (*55, 57, 58, 265*).

Other applications to the study of proteins (*8, 161, 189, 203*) include microabsorptiometry of protein and iodine in the colloid of individual thyroid follicles (*123*) and the absorption of virus protein (*264*) which is also a nucleoprotein. Thiouracils (*102, 279*) and estrogens (*116*) have also been investigated by means of ultraviolet absorptiometry, as have various hormones (*203*), fats, fatty acids, and oils (*18, 43*), iodine (*85, 267*), and vitamins (*8, 134, 225, 259, 278*). A large number of organic solvents were studied by Maclean, Jencks, and Acree (*202*).

Infrared Absorption. The very large and important field of infrared absorptiometry has developed rapidly during and since the last war, largely because of its unique contribution to the fields of hydrocarbon and synthetic rubber chemistry. Both because of the rapid development of the field and the scarcity and expense of infrared spectrometers, the biochemist has tended to neglect this field. When it is considered that the emission and absorption of infrared radiation depend primarily on the types of bonds in and between molecules, it is apparent that the ultimate value of the technique may well be comparable to its tremendous importance in the petroleum industry. Necessarily, the interpretation of the spectra of the complex materials of the biochemist will be far more difficult than is true for the relatively simpler hydrocarbons. For this very reason, the technique offers more ultimate value than would the simpler approach capable only of restricted though easy interpretation.

No effort will be made to survey the literature of the field except for those applications which have been made directly to biochemistry. The total number of publications in the general field runs into many hundreds and is increasing rapidly. It was reviewed recently by Barnes and Gore (*16*), who quote numerous earlier reviews. The development of suitable equipment has taken place relatively recently, and rapid progress is still being made. The degree of opacity of most glasses to infrared radiation is such as to force the use of unconventional materials. Ordinarily prisms of sodium chloride crystal are used as dispersion devices, but prisms of several other salts are now available (*16, 17, 157*). Absorption cells (or windows) also must be constructed of such materials, most of which are soluble in water. This, added to the strong absorption of water in the infrared, makes difficult the use of aqueous systems, and constitutes the most serious limitation to general biochemical application. However, silver chloride (*13, 128*) has proved valuable for window construction, and now even aqueous systems are susceptible to limited study. Much progress has also been made in the development of better infrared-sensitive photometric equipment, and there are indications that lead sulfide photocells (*184, 282*) and vacuum thermocouples (*16*) will shortly replace the older devices, which often were simple thermocouples. Technique is constantly being simplified and improved (*160, 239, 266*). The field of polarized infrared (*4, 5*) is also largely undeveloped and should be definitely applicable to biochemical studies of several types, because it should make possible the determination of the orientation of groups that are infrared-active in solid phases.

Infrared absorptiometry has been applied to qualitative and quantitative analysis of nucleic acids and their derivatives (*29*), steroids (*91, 119*), amino acids (*175, 186*), protein and peptides (*4, 5, 38, 49, 174, 186*), tocopherols (*254*), fatty acids (*247*), and a large number of specific compounds, groups of compounds, and types of bonds not directly of interest in biochemical investigation. An interesting application was made by Blout and Mellors (*30*) who determined the absorption spectrum of tissue sections and blood smears. They assigned the absorption chiefly to protein and correlated it with the various bonds which were stretched or bent.

Raman Spectroscopy. This technique is related in its applications to infrared absorptiometry, though it is itself not an absorption method. It depends on measuring the spectrum of the light scattered at 90° by a material in solution when the latter is exposed to a monochromatic source of high intensity and relatively short wave length. This property is comparatively specific for chemical groups of several types. It will distinguish at times between cis- and trans- configuration, as in oleic acid which is cis and elaidic acid which is trans (*95*). It will locate and distinguish double and triple bonds and many specific groups. It is therefore a technique which has a large potential biochemical use, largely unrealized because of the scarcity and cost of the equipment. The general field has been recently reviewed by Braun and Fenske (*41*), while Goubeau (*129*) and Gerding (*122*) have discussed its application to the analysis of organic compounds. Renard (*250*) applied it to the study of amino acids in protein hydrolyzates. A fairly large body of literature (*41*) is available on the application of Raman spectra to the analysis and study of natural products, largely in the petroleum and related organic fields.

X-Ray Absorption. To the best of the authors' knowledge, x-ray absorption measurement has not yet been directly applied in the field of biochemistry. Its inclusion in this review is justified only on the ground that it is now a reasonably well-developed technique which unquestionably has potential applications in biochemistry and will ultimately make significant contributions to that field (*191*).

X-ray absorption does not overlap significantly on the other fields of absorptiometry because the x-ray is of so much shorter wave length that its absorption is by the electrons close to the nucleus rather than those in the outer shells, as is true of light absorption. The character of the absorption is then a property of the element, not of its chemical form. Because this is the case, x-ray absorption may be used to determine the total of an element in a mixture without regard to its compounds. The same amount of oxygen in glucose and in quartz will show the same type and amount of absorption. The biochemist is less concerned with elements as such than with compounds, except as a final determinative step of his analysis. However, there will certainly be many instances in which such an over-all analysis of a single element will be most useful in evaluating a biological system.

While x-ray absorption measurements were made as early as 1925 by Glocker and Frohnmayer (*127*), it is the recent developments by Liebhafsky *et al.* (*192, 193*) of a multiplier phototube photometer (*323*) that have made relatively practical its general use as an analytical tool. Further developments such as that of Frevel and North (*191*) of automatic recording equipment along with an x-ray monochromator will unquestionably extend greatly the general utility of this technique. Pavelka and Gentile (*229*) record the use of x-ray absorption in determining small quantities of inorganic salts.

ISOTOPE TRACER TECHNIQUES

The ability to label an element, either through use of an isotope having a mass which is different from the normal, or one that is radioactive, has been of the greatest value in biochemistry. It will certainly remain for a long time a primary research technique in this field. It must, therefore, take an important place in any discussion of biochemical analysis. As with many other generalized techniques, it is so broad and detailed a field as to defy complete review within the scope of this paper. Its proper place in the general biochemical analysis picture may be indicated, along with some of its more important uses, advantages, and limitations.

Isotopes which have a mass different from that of the plentiful isotopes—e.g., H^2 or D, C^{13}, O^{18}, or N^{15}—have been utilized in biochemical studies for a considerable number of years (*201, 263, 304*). They are very effective in this field because these elements are of general occurrence throughout the animal and plant kingdoms and are found in most of the compounds present there. These heavy isotopes are difficult to use because there is no simple method of detecting them. Ordinarily this is accomplished by isolating the compounds suspected of containing them, subjecting them to combustion, and analyzing the resulting gases with the mass spectrometer (*309, 314*). This technique has led to very important advances in biochemical knowledge (*1, 45, 96, 112, 163, 164, 270, 271, 291*) and promises still further contributions because of the lack of suitable radioactive isotopes of hydrogen, oxygen, and nitrogen. Labeled isotopes of carbon are more effective than those of the other elements mentioned because of the lability of groups containing the latter and the fact that the metabolism of the carbon chain rather than its attached groups is the fundamental problem.

Much more far-reaching in effect is the use of radioactive tracers, because these isotopes may be readily detected and estimated by means of one of a variety of counters (*117, 120, 166, 209*). Most valuable perhaps is C^{14}, a weak β-ray emitter with a long half-life. In spite of the weak radiation which makes assay difficult, this isotope is far easier to use than the heavy stable isotope C^{13}, or the short half-life C^{11} which yields a considerably more energetic emission. Other isotopes which have great importance in general biochemical studies are P^{32} (*47, 72, 166, 256*) and S^{35} (*144, 166*), both of which are readily assayed. P^{32} is highly significant because of the wide chemical distribution of phosphorus in the proteins, nucleic acid (*143*), phospholipides (*61*), and phosphate esters of carbohydrates and their metabolic products. S^{35} is useful in following the metabolism of the sulfur-containing amino acids, cystine, cysteine, and methionine (*212, 287, 288*), and other compounds such as glutathione which have significant roles to play in oxidation, enzyme reactions, and other biological mechanisms (*144*). Somewhat less broad in their applications, but significant in biochemical study, are the radioactive isotopes of iodine (*183, 289, 290*), iron (*77, 124*), sodium and potassium (*110, 144*), cobalt (*144*), arsenic, and others, the parent elements of which are not major constituents of animal and plant materials.

In a sense, isotope investigation is a fractionation, in that it can serve to distinguish a compound carrying a labeled from one carrying an unlabeled element, thus often indicating synthesis and metabolic rearrangement. Its chief value, however, is to indicate the pathways of metabolism, thus telling "what is formed" and "how much." In order to accomplish this, every isotope study requires chemical fractionation. No radioactive indicator can distinguish the chemical form of the element detected. Thus, the chemical separation is in a sense more critical than the tracer itself, because the extent of the information is limited by the fractionation rather than by the presence or amount of tracer detected. A reciprocal relation exists between the chemical and the radioassay phases of the study. The tracer may be used to determine the efficiency of fractionation, and the fractionation limits the utility of the tracer. Thus tracer study is a supplement to chemical fractionation, and may be considered as a determinative step to follow the preparative chemical fractionation step.

As compared with chemical determinative methods, the tracer determination is usually considerably less accurate. Counting assay, itself, may be performed only rarely with the accuracy and precision of good chemical analysis. If to this are added the technical problems of control of self-absorption, coincidence, geometry, and the occasional vagaries of electronic counters, it is clear that quantitative accuracy is not always obtained. As a comparative method, even these uncertainties and errors are rarely of sufficient magnitude to invalidate conclusions based on tracer study.

As a supplement to the simple tracing of a labeled element by means of radioactivity or mass difference, the use of isotope dilution analysis (*147*) has been valuable. Heavy isotopes may be used to obtain the elementary composition of a pure compound by adding a known amount of the unusual isotope in any convenient form to a known amount of the pure compound, and heating above redness until equilibrium is reached. The resulting mixture is analyzed in the mass spectrometer to obtain the isotope ratio (*258, 309*). Quantitative recovery of the material is not necessary. A more valuable isotope dilution method for biological use consists in adding to a mixture a known amount of the pure compound in question which contains the labeled isotope, and isolating some of the compound in question in pure form from the mixture. Measurement of the labeled (usually radioactive) isotope in the isolated material as compared with the total material isolated gives the ratio from which the original compound in question may be calculated. Here also, quantitative recovery is not necessary.

Another valuable application of radioactive tracers is the radioautograph technique. Here a tissue section—e.g., bone, leaf, etc.—suspected of containing radioactive material is placed in apposition to a sensitive film or stack of films and allowed to record its distribution by blackening of the film (*25, 39, 273*). Less valuable than the other methods mentioned for quantitative use, it does record distribution with respect to the large microstructures in a simple and effective manner.

The general field of radioisotope analysis is covered in a number of selected books and reviews (*50, 51, 117, 145, 166, 304*). Because of the pre-eminent position of the radioactive carbon isotope, C^{14}, in biochemical investigation, some illustrations of the versatile applications of this tracer are indicated. No effort is made to do more than illustrate the possibilities. The metabolic products derived from labeled molecules, and precursors of compounds of interest are uniquely determined by the carbon tracer studies. Thus, β-labeled tyrosine has been shown to yield α-labeled acetoacetate; phenylalanine to yield epinephrine; glycine to yield serine; β-labeled tryptophan to yield nonlabeled nicotinic acid; and palmitic acid labeled in the C_6 position to yield glucose (*130, 138, 248, 304*). Interesting and significant studies of the mechanism of photosynthesis were made by Calvin *et al.* (*25, 273*), while Heidelberger *et al.* (*137, 139, 140*) studied extensively the distribution and excretion of benzanthracene. Innumerable examples might be quoted, but the reviews of Bloch (*27*), Wood and Lorber (*318*), and Vennesland (*304*) serve to illustrate further this important question.

RESPIROMETRY

An essential to the biochemist is a knowledge of the total metabolism of his living system. This is well measured by the rate of oxygen consumption in terms of some fundamental unit of mass, volume, or surface of the respiring system, and is better evaluated in terms of both oxygen consumption and carbon dioxide production, or the respiratory quotient defined as CO_2/O_2. With larger animals and whole plants of reasonable size, the techniques of obtaining these values have long been known and used. Such information is valuable when it concerns the intact animal or plant, but can only represent an average value for all the cells and types of cells that are respiring at different rates and the inert, nonrespiring portions of the organism. Much more information is available from studies of uniform tissues, small organs, and single cells or multiple homogeneous cells. The field of microrespirometry, covering this type of material, is much newer and of greater continuing research importance. The discussion of respirometry will for this reason be limited to its small scale aspects suitable for measuring the metabolism of the small biological system.

Barcroft-Warburg Technique. The well known constant volume manometric apparatus associated with Warburg (*308*) and adapted from the earlier equipment of Barcroft and Haldane (*15*) is too widely employed to require detailed review here. The technique has been discussed in detail in several publications (*14, 90, 230, 302*), and has been used as a tool of major importance in a wide variety of biochemical investigations.

The most important feature of this equipment and technique is its versatility. The reaction chambers may be designed to add at will any reagent desired in an appropriate quantity and at any time. This allows serial experimentation on the same biological material. The chief requirement of the method is that the volume (or pressure) of the gas phase be altered by the reaction. The considerable sensitivity of the instrument to alterations in pressure, temperature, solubilities, and reaction rates imposes limitations on its precision and accuracy. The use of the Warburg apparatus is not restricted to respirometry as such, and in fact its chief uses are in the study of enzyme systems, biological mechanisms, and related matters with tissue slices (*107*), homogenates (*243*), partial homogenates (*187*), and isolated enzymic materials (*219, 281*). No gasometric or respirometric equipment has exceeded the Warburg technique in the variety of applications. Several have exceeded it in sensitivity and some in accuracy.

Smaller respirometers capable of accomplishing similar results to the Warburg apparatus, but with smaller systems not in the single cell range, have been described. One of the more ingenious was that of Heatley, Berenblum, and Chain (*136*), which operated by means of a flexible mica window attached to a sensitive optical system which registered any alteration in pressure in the respiration chamber.

Differential Microrespirometry. Differential manometers have long been applied to respirometric measurements (*294*). They have the advantage of making it possible to eliminate some of the sources of uncertainty in the standard Barcroft-Warburg apparatus, such as sensitivity to temperature fluctuation, barometric pressure changes, and variables in the chemical or physiological system. Fenn (*106*) devised one of the early practical differential respirometers which has found wide application. Constructed as it was of glass, it was somewhat bulky and subject to local temperature changes. Its range of operation is limited in comparison to the Warburg instrument, the sensitivity being 3 to 4 cu. mm. per centimeter of scale, or approximately 5 times as sensitive as the Warburg apparatus. Summerson (*280*) described a differential respirometer having nearly all the features of the Warburg apparatus and about the same size and sensitivity. It consisted essentially of two Warburg units attached together through their manometers. A considerably more sensitive device developed by Thimann and Commoner (*293*) required careful thermostat control of temperature and accessory mounting apparatus. All the above instruments fall short when applied to the truly small biological system.

Cunningham and Kirk (*84*) described a much simplified type of differential microrespirometer that was constructed in a single block of metal, thus reducing the requirements for temperature control to a minimum. It was independent of barometer changes, and extraneous experimental variations could be controlled through the second chamber. Furthermore, it was more sensitive than other differential instruments—i.e., 0.1 to 10λ per hour. Modifications by Barth and Kirk (*22*) and by Stern and Kirk (*274*) have further improved its operation, so that it is probably far simpler than any other comparable instrument. It can be applied through a wide range of sensitivities and to almost any type of biological system. It further had some of the advantages of the Warburg apparatus, in that a second reagent could be added during a run, and the gas phase could be modified at will. A modification of this instrument for insect respirometry was published by Smith and Douglas (*268*).

Ultramicrorespirometry. Measurement of respiration of the smallest systems, including single cells, has developed markedly in recent years, and significant progress has been made in applying the instruments to the more general type of study as is characteristic of the Barcroft-Warburg apparatus. Fine capillary respirometry as initiated by Kalmus (*165*) and Howland and Bernstein (*156*) was significantly improved by Tobias and Gerard (*299*) and particularly by Cunningham and Kirk (*83*) who achieved accurate measurements with a sensitivity of $5 \times 10^{-5}\lambda$. This type of instrument is inherently limited to respiratory measurements rather than general biochemical application.

From the standpoint of general use, the Cartesian diver manometer (*195, 196*) has almost unlimited potentialities, some of which have been realized, and toward which progress is constantly being made. The technique of the Cartesian diver as applied to respiratory and biochemical studies has been covered in several publications (*126, 154, 170, 230, 323*). Its theory is given in detail by Linderstrøm-Lang (*194*), and some of its most important uses have been developed by Boell, Needham, *et al.* (*31-33*). More recently it has been used for weighing minute amounts of tissue or cells (*325*), an operation which is less sensitive than some other weighing methods (*171, 172*), but is peculiarly adapted to the need for keeping the tissue immersed in an aqueous solution. Anfinsen and Claff (*7*) and particularly Claff and Tahmisian (*65*) have adapted the design of the diver to allow general biochemical application in the sense that the Warburg apparatus is employed. Numerous simplifications, for the most part not yet published, in the construction and operation of the diver, have been achieved (*170, 324*). It appears highly probable that the diver, and perhaps the differential respirometer as well, will serve to elucidate composition and mechanisms in smaller and more homogeneous systems than are available for study with the Warburg apparatus. The sensitivity of the Cartesian diver, such as to make possible this extension to single cells, thus expands greatly the possibilities of biochemical research with the smallest biological units.

The subjects discussed above represent only a few of those that might have been chosen for review. They should serve to indicate the increasing breadth of the field as well as some of the progress made in recent years. Above all, it is hoped that the discussion indicates some of the basic considerations that must be a part of the biochemist's philosophy if he is to apply to his problems mature judgment in the choice of method.

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[End of Review Section]

Automatic Operations in Quantitative Analysis

GORDON D. PATTERSON, JR., WITH M. G. MELLON
Purdue University, Lafayette, Ind.

Automatic analyses are discussed from the point of view of the individual unit operations involved in any general method of analysis, as applied to the sample and the desired constituent. Inasmuch as the major attempts to automatize industrial analyses have been in the line of measurements of physical properties, greatest emphasis is placed on these developments. Methods using physical properties for automatic measurement are classed under one of the following topics: gravimetry, volumetry, densimetry, manometry, viscometry, magnetometry, titrimetry, calorimetry, chronometry, sonimetry, emissimetry, absorptiometry, thermoconductometry, turbidimetry, refractometry, diffractometry, reflectometry, conductometry, potentiometry, currentimetry, dielectimetry, and radioactimetry.

THE phenomenal growth in recent years in the automatization of unit operations and/or entire methods of quantitative chemical analysis indicates a rapidly accelerating "industrial revolution" in the field of analytical chemistry. It might well be said that this trend is also in a direction away from the chemistry in analytical chemistry and toward identification and measurement by strictly physical means. This is because many types of physical measurements, particularly those involving electrical aids, frequently lend themselves readily to continuous indicating and/or recording. It is this goal toward which all industry seems to be heading in the solution of routine analytical problems (*35*, *91*, *164*).

The question of when a method is automatic and when it is not is interpreted differently by different people. At the present time a large number of instruments are being designed and used which may perform only limited parts of the over-all analytical process (*367*). If one considers a method to be a composite number of unit operations, any of which may have automatic possibilities, resulting in "semiautomatic" methods, then it is easy to see the impracticability of placing an arbitrary border between manual and automatic methods. Actually, a strictly automatic method completely eliminates the requirement of the human hand or judgment and performs everything from obtaining the sample to recording the results desired. Therefore, the term "automatic operations" is used in this paper to accentuate the fact that complete analyses are rarely automatized as such and to include those methods where essential operations have been automatized even though the entire process may in part still require manual assistance (*140*).

The factors that have prompted this industrial trend toward automatic analyses are basically two: a need for mountains of data which would be unattainable manually; and a desire for increased precision and accuracy at lower cost, by minimizing in so far as possible the human variable as one of the potential sources of error. This emphatic shift in methods development, wherein there is required familiarity with the design, construction, and operation of instruments, has made "analytical chemists" out of many scientists in various other fields. This study has the purpose of ascertaining just what basic factors have contributed to, and indeed made possible, this replacement of the routine analyst as a data gatherer by the instrument, and of describing methods and operations that have been automatized.

The three factors which industry considers in obtaining any analytical information are amount of information, cost of the information, and speed of obtaining it. These three are not wholly independent of each other, but in general automatic methods, when properly adapted to routine work, can be superior to manual methods in all three respects. However, there are also three possible disadvantages: The potential variety of uses of an automatic method varies inversely with its degree of automatization; lengthy and costly development work is frequently required in setting up a new method; and maintenance troubles vary directly with the amount of gadgetry involved (*230*).

The contribution of electronics (*100*, *345*) to modern analytical instrumentation has been so notable that vacuum tubes have been called the "brain cells of modern technology." At a recent Symposium on Modern Methods of Analysis (*86*) a remark was made that there was not a single mention of a buret or crucible on the entire program, that "after all, one just has to have some electronic mechanism in an apparatus these days," and that the modern analyst must be half physicist, half electronics engineer, and all chemist (*97*, *98*, *99*).

Most studies of autoanalysis have been limited to more or less specific principles and applications. A generalized study and classification giving an over-all picture of the field have not appeared. On the other hand, automatic methods are not something that suddenly appeared from nowhere.

The literature reveals that instruments of automatic measurement are designed on one of two general bases: mainly an adaptation of a classical method previously performed manually, or an application of a new technique relating measurements, which are more readily automatized, to the information desired. In considering an automatic method, therefore, it seems most logical to outline the steps involved in the same way as would be done in the case of any classical analytical method. The automatic methods described are classified according to the outline presented elsewhere (*339, 340, 390A*).

THE SAMPLE

Primary consideration in any analytical method should be a representative sample. Unless due care is taken in considering the various factors of importance in obtaining a representative sample, the most expensive and complicated measuring equipment becomes useless.

Three operations may ordinarily be performed on the sample in any manual analysis: selection and preparation, measurement, and preliminary treatment. However, few automatic methods contain provisions for all three. Whenever possible, preparation and preliminary treatment are avoided; but the two unavoidable sampling operations are selection and measurement.

SELECTION AND PREPARATION

Selection usually consists in locating a sampling point and in transporting the material to the point of measurement. In general, automatic sampling of freely flowing materials can be either intermittent or continuous. The material that is being sampled must, of course, be homogeneous, especially if only one sampling point is used. It may be of changing composition, but must be at least representative of the state of the system at any one time. When necessary, several points in the system will be chosen as sampling locations in an attempt to obtain an over-all result and eliminate variations within the total amount of substance in question.

Operations involving preparation are ordinarily not required in gaseous, fluid, or homogeneous solid samples, but the problems encountered in nonhomogeneous solids, such as grinding, mixing, and sieving, may be handled semiautomatically, by means of machine-operated crushers, ball mills, mortars, and mixers.

When a gaseous or liquid sample must be transported to a remote measuring location, a by-pass line is frequently used. Care must be taken to ascertain the effects of various factors, such as temperature and other conditions, which may cause false measurements—for example, conductometric measurements of water are greatly influenced by carbon dioxide content. Large time lags are generally avoided.

Various devices have been used for the intermittent sampling of gases (*4, 20, 39, 79, 215, 217, 222, 294, 300, 302, 352, 398*) and liquids (*41, 111, 166, 192, 288, 291, 317, 358, 405, 434, 496, 517*). One apparatus recently described for sampling gases collects at regular intervals bubbles of the gas (*436*). An apparatus (*427*) "for automatically periodically segregating measured samples of liquids" is used in testing water hardness in softening operations. Another, used for sampling juices in a Brazil sugar factory (*364*), consists of a float which actuates a vertical rack and gear to which is attached a lever bearing a small bucket. As the measuring tank fills, the bucket is lowered into a receptacle. A specialized sampler (*455*) for explosion gases functions automatically from the impulse or the heating effect of the gases.

A novel sampler (*88*) which will collect automatically up to 200 portions from a laboratory column is suitable for low take-off fractionating or chromatographic columns. It consists of a platform on which the receivers are arranged in circles and depends on a photoelectric cell to count the drops and rotate the platform when the proper number have fallen into a given collecting vessel.

In collecting solid samples (*250, 292, 526*), scooping off conveyer belts is a reliable method. Various other proportioning devices have been described for quickly separating a small representative sample from a large mass of material passed through. In an apparatus for collecting and segregating samples of crushed coal or coke (*411*), a thin stream runs from a hopper into twelve triangular radially shaped receivers mounted on a rotating table. If the bulk sample takes 5 minutes to run from the hopper, the sample in each receiver is made up of 300 increments selected without bias from every part of the bulk. Another commercially available sampler (*165*) has been successfully applied in many industries for a wide range of wet and dry materials (*284*).

For solids suspended in gases, such as dust in air (*118, 430, 473, 537*), various devices are in use, ranging from filters (*417*) to adhesive or impregnatable surfaces (*325, 432*). An "electrical dust precipitator" (*387*) makes use of a steady stream of liquid washing down over the negative plates of an electrode pair to remove continuously the dirt precipitated on them. In practically all industrial hygiene studies and investigations, air sampling of contaminants (*183, 470, 471, 472*) is a necessary part of the procedure. Special devices (*307, 463*) have been designed for a few of these contaminants for determining their concentrations directly in the field.

In connection with measurement of radioactive properties an automatic sample changer (*516*) is used to speed up counting measurements by means of a rotating circular tray.

MEASUREMENT

Measurement of the sample may be controlled in various ways if an automatic sampler is used. The material is frequently confined in a given volume (*224*) under constant conditions and flushed or drained out periodically. When possible, the dimensions of the container or measuring element, together with the rate of flow of the gas or liquid, will control the sample amounts—e.g., cell thickness in the case of radiant energy transmission, or area and interval of the plates of an electrical conductivity cell. Obvious sample measurement is often lacking, as in the case of a pH meter, but in all cases it is vital in the calibration and standardization procedures. Of the two common properties used in sample measurement, mass and volume, the latter is the one most resorted to in automatic operations (*9, 198, 278, 305, 326, 427, 564*).

If the sample measurement is to be made by constant velocity of flow (*55, 172, 218, 306, 425*), there are various means of assuring such constancy, most of which are based on pressure uniformity. When electric, motor-operated pumps are used, line voltage variations cause especially serious errors at low speeds. A flowmeter designed by the Department of Public Health of Illinois, meeting the following minimum specifications, has been described: Air flow should be at least semiautomatically controlled; it should be simple in construction and at the same time rugged and easy to operate; it should be applicable to both low and high sampling rates with equal accuracy; parts should be easily accessible in the case for cleaning and repair; and it should be light in weight for maximum portability.

Occasionally, as in measurements dependent on thermal conductivity, readings may be relatively independent of the rate of sample flow up to a certain limit. In the Magnotherm continuous oxygen recorder (*215*), a pressure compensator is incorporated which works into the recorder circuit to correct for changes in pressure, inasmuch as the instrument measures actual partial pressure of oxygen and not the percentage by volume as is required for general gas analysis work.

When actual volumes of gases are measured, the procedure consists of means of getting a sample into a container and flushing it out by a piston plunger action, frequently with mercury. For liquids and granular solids a common procedure makes use of the "mill-wheel" method (*269*) and variations of it. Numerous constant-feed devices are also on the market. A typical volumetric method of measuring solids (*21*) consists of a curved metal vane projecting vertically down the center of the spout through which the sample moves, thus causing the vane to rotate a definite amount with each foot of sample travel. This rotatory motion is transferred through a flexible shaft to the counter.

A number of drop counters have been used for sample measurement which may be photoelectric or conductometric in operation (*49, 202, 208*).

PRELIMINARY TREATMENT

Automatic methods eliminate preliminary treatment whenever possible. Adjustments of conditions (*361*) such as pH, temperature, pressure, water-vapor content, and oxidation state may be necessary, however, and have been included in various methods. Constancy of temperature and pressure in the cases of gases, as well as liquids, is an obvious precaution not always easily obtainable in practice, and nearly every method designed must include study of the influence of these factors. Water vapor saturation and oxidation of gass amples frequently are factors with which to be dealt.

THE DESIRED CONSTITUENT

Once the sampling problem is solved, the next point to be considered is always the desired constituent. Here the two fundamental operations are separation and measurement; the former is to be avoided if possible, and the latter is the goal to which all other operations contribute.

SEPARATION

Five major types of separation have been subjected to a number of recent studies of their automatization possibilities.

1. **Precipitation.** Precipitation, as a separation procedure at least, is an operation in which no attempts at complete automatization have been published in spite of the fact that separation by precipitation and filtration, which so frequently precedes gravimetric measurement, is one of the most common fundamental operations of classical methods of analysis. The operation of adding precipitant until there is completeness of reaction might conceivably be made by the use of the potentiometric titration apparatus mentioned below, but the operations required in handling the precipitate, such as digesting, filtering, washing, and drying (or igniting) do not lend themselves readily to being automatized.

Automatic filtration (*30, 501*) is usually based on a system wherein quantities of liquid are brought to the filter equal to those flowing away and the surface is therefore kept at a constant level.

2. **Volatilization.** Volatilization as a means of separation has recently received attention in the construction of automatic stills and fractionating columns (*130, 480*), particularly in work with petroleum and hydrocarbons in general. An automatic distillation apparatus (*435*) for A.S.T.M. Method D-86 automatically plots a complete distillation curve. The operator simply fills the distillation flask with the sample, inserts the chart paper, and sets the initial heat, whereupon the instrument performs the distillation, records all required data such as correction temperature, initial boiling point, end point, and distillation rate, and then automatically resets itself for the next test.

The Podbielniak apparatus permits the quantitative separation of hydrocarbons in a single fractionating operation. The procedure involves freeing the gas of water vapor and carbon dioxide, liquefying the sample, and introducing it into the evacuated system in which the distilling bulb is cooled by liquid air. In the Turner modification a heater at 750° F. gradually runs up the 6-foot column in about 8 hours. At the same time a rising mercury column drives out the liberated gas to a thermal conductivity cell and the "volumeter."

Many units for automatic combustion of organic substances have been described (*332*). Use of automatic combustion methods has been made in the Dumas determination of nitrogen (*565*) and also in the Pregl determination of carbon and hydrogen (*95, 229, 566*), in which the tube containing the sample (solid, liquid, or gas) can be heated electrically little by little.

3. **Electrodeposition.** Separation by electrodeposition by an externally supplied potential has received some attention from "automatists." The so-called graded cathode potential method for various metals has been reviewed recently by Diehl (*126*). It affords the separation from each other of two metals which lie only slightly apart in the electromotive series—e.g., copper and tin—by means of constant applied potential rather than constant current. Besides the Diehl apparatus, circuits by Hickling (*220*) and Lingane (*316*) have been described. Fisher (*153*) has described the use in atomic research work of a voltage regulator designed by Pickle, which is a servomechanism incorporating a direct current amplifier and motor drive for furnishing constant potentials as great as 12 volts and currents as great as 1 ampere under load conditions that change several hundred per cent in 24 hours. Three electrodes are used: a cathode, probe, and anode.

Latest commercial apparatus (*412*) making use of the principle of separating interfering constituents with mercury cathodes is the "mercalyzer" for rapidly removing heavy metals from aqueous solutions of reactive metals.

4. **Extraction.** Various automatic extraction procedures have been devised, but few have received widespread attention. Some use of dissolution as a separative procedure automatically has been made. Methods wherein a continuous solvent is caused to flow slowly over the sample, only part of which is soluble in the liquid, employ so-called continuous extractors such as the Wiley, Bailey-Walker, and Soxhlet extractors.

A large-capacity continuous solids extractor (*11*), designed by Appelzweig, holds the sample in a large aspirator bottle from which the bottom has been removed and uses constant percolation instead of repeated exhaustion as in the Soxhlet types.

Absorption has seen application in several automatic gas analysis methods (*330*), notably the reaction of carbon dioxide in potassium hydroxide solutions where volumes or pressures of the sample are measured before and after absorption.

Adsorption has been similarly applied, particularly in various forms of automatic combustion trains. The Turner-Burrell adsorption fractionator (*71*) automatically separates components of light hydrocarbon gas mixtures by distilling the liquefied sample and, instead of condensing the various fractions, adsorbing them on an adsorption column. The adsorbed gases are then desorbed from the column packing by means of a heater which slowly travels up the column and a pool of mercury which displaces the gases upwards. The volume and thermal conductivity of the effluent gases are then measured. Chromatography is finding expanded usage, and it also has been the basis of separations where the flow of a liquid through the adsorbing column can be controlled automatically.

In partition methods, where a constituent is transferred from one liquid medium to another (which is immiscible with the first) by virtue of its differing solubilities in the two, various ingenious apparatus have been described, most of which are analogous to the above-mentioned continuous extractors. Continuous liquid-liquid extractors of varying modifications have been described recently (*270, 393, 447, 515*). Wayman and Wright (*539*) described several small continuous liquid-liquid extractors wherein samples as small as 5 ml. can be used.

Dialysis and ion-exchange methods have not found sufficient application on a routine basis for analytical work to induce automatization, although continuous dialyzers are not unknown (*273*). A recent type for use in biochemical problems (labeled the "artificial kidney") combines a large cellophane area for small amounts of solution with a countercurrent design which makes for greater efficiency and speed. Kolff and Berk (*271*) describe its use for discontinuous and continuous dialysis of blood in an arrangement where the blood flows from a vein through a narrow cellophane tubing, wound around a wooden drum that rotates in the dialyzing bath. The blood then returns to the circulation system of the body, after having been exposed to a surface of 20,000 sq. cm. in a relatively short period of time. The Webcell continuous dialyzer (*60*) has a Lucite construction which makes visual observation possible. Automatic ion exchangers for water purification purposes have been described and their extension to separations in analytical methods should certainly be possible.

5. **Magnetization.** Use of magnetism as a separative force (*458*) is made mainly in the so-called mass spectra procedures (*53, 87, 102, 378, 415, 437, 441*). The separation is attained by ionizing the gas by electron bombardment, accelerating and focusing the ions by an electrostatic field, and then deflecting them in a magnetic field. The separation of the ions according to their masses is thus accomplished and abundance measurements are then made electrically (*320*) or photographically. One process control spectrometer (*175, 293*) programs a series of eight accelerating voltages corresponding to specific preset masses to be separated.

Magnetic separations such as are used in refining ores passing off conveyer belts may be used as a basis of rough evaluation but can hardly be called quantitative (*128*). One magnetic separator is of the submerged belt type (*109*) with means of projecting wash liquid sprays onto the traveling belt to sweep down the nonmagnetic particles.

MEASUREMENT

The climax of any analytical method is the measurement operation by which the actual quantity of the desired constituent present is determined, and this phase of quantitative analysis has received the most attention in efforts toward automatization. The following discussion of automatic methods of measurement is arranged in accordance with the outline presented in another paper (*390A*).

Gravimetry. The term "gravimetry" is limited to the mass (or weight) measurement of the desired constituent or of some system directly related to it, although mass measurement of the sample involves the same operations (*190, 197, 343, 454*).

The equal-arm balance whose beam is supported at one point along its length, so that two masses, suspended from the two ends, may be compared by noting the swing of a pointer, is one of the oldest measuring instruments on record. It is also one of the fastest means of accurate measurement in existence; yet in routine work it is time-consuming and so frequently used that accurate automatic weighing devices are highly to be desired.

One adaptation (*448*) of the classical analytical balance for such purposes makes use of the interruption by the pointer (or an opaque screen attached to it) of a beam of light, so that varying deflections cause corresponding variations in the amount of light reaching a photocell whose photoelectric response can be measured by a microammeter.

Various automatically recording balances for studies of processes where weight changes occur have been developed (*144, 503*). They may have a motor-driven chain for restoring balance or may have a recorder pen supported on the end of the balance beam. Moisture studies (*2, 188, 371, 533*) have made use of recording balances. A semiautomatic commercial device for moisture in coal, subject of a recent study by the Bureau of Mines (*51, 104*), is a combination of a drying oven and balance with a capacity of ten 10-gram samples. The weighings are made without removing the samples from the oven or disturbing the drying process. The percentage of moisture removed from the coal is indicated by an illuminated dial when a definite weight of sample is used in a tared pan. An automatically recording electrical sorption balance (*188*) graphs time versus change of mass of a solid adsorbent or reactant. As the mass changes, the balance beam is automatically brought to its null position by alteration of the current flowing through a control solenoid.

Müller's "electronic recording analytical balance" provides for substitution of inertia- and lag-free electronic methods for electrical contactors and relays to provide a recording, direct-reading rapid sensitive balance which is suitable for either slow or rapid weight changes.

Radioactive detection of displacement (*45*) also shows promise of use in this field. When radioactive material is coupled directly or indirectly to the moving part of a system, variations in position cause variations in ionization current or light effect that are readily amplified to give practically any sensitivity and precision. Patents by Blau and Feuer (*45*) describe the possibilities of this means of measuring mass. They also describe a radioactive limiting balance for production weighing of small quantities of material within a predetermined accuracy. Here, two radioactive foils and ionization chambers are attached at opposite ends of the balance beam so as to give a maximum reading when a predetermined weight of material is on one balance pan. With high enough radioactive intensities, or increased amplification in the output circuit, it would be possible to energize a relay controlling a valve that would permit only a certain amount of material to flow onto the balance pan.

Replacement of the equal-arm balance design by a single tray supported in space by an electromagnet, part of whose current is controlled by the position of the moving system, has been made (*94*).

> The problem of converting this position into a voltage to be amplified and fed into a winding on the magnet can be accomplished in one of four ways: (*a*) interruption of a light beam on a photocell, (*b*) capacitance variation in condenser plates caused by a vane moving between them, (*c*) inductance variation of a coil caused by an iron core attached to the moving system, and (*d*) variation of Q (ratio of reactance to resistance) of a coil by an iron core as in (*c*). The possibilities of (*a*), but not of the other three, have been tested by Clark (*94*). Precision equal to the beam-type balance can be obtained without imposing unreasonable requirements on the electronic components, while the sensitivity may even be superior. In the model built the output of the photocell is amplified and connected to the electromagnet winding so as to oppose any motion of the suspended system. The object to be weighed is placed on the tray and the additional current required to restore the system to its equilibrium position is read on a meter.

An instrument having the balance beam under constant load has recently been placed on the market (*156, 157*) to serve as a high-speed routine analytical balance with direct-reading operation from 200 grams to 0.1 mg. at uniform sensitivity throughout the entire capacity.

> In operation, remote control levers connected to direct-reading dials remove weights from the pan side of the beam to compensate for the added mass placed on the pan. Having only one pan, the balance beam has the other end loaded with a constant tare weight. The portion of the weighing under 100 mg. is taken directly from a projection of a scale on a beam to a stationary vernier scale through an optical system.

A recording microbalance for measurement of vapor diffusion through plastic membranes (*533*) has been designed by placing a water-absorbent pellet at one end of a glass balance beam which passes through the center of a coreless lacquered rotating coil, both of which are suspended horizontally by a torsion band. Two large coils outside the vessel induce currents in the rotating coil when the glass beam moves out of its equilibrium position owing to absorption of water that has passed through the membrane. This current restores the system to equilibrium and operates a pen recording the change in weight per unit of time from 10 micrograms up.

Duval has conducted studies on automatic gravimetry (*137, 138, 394*), using the Chevenard thermobalance which gives automatically a photographic record of the weights of ignited inorganic precipitates (*251*). Pyrolysis curves of a number of analytically useful precipitates were determined. A paper by Peltier and Duval (*394*) gave directions for "the completely automatic analysis of some alloys of silver and copper." By means of the thermobalance the progressive loss in weight of a mixture of copper and silver nitrates with increasing temperature was automatically recorded.

Volumetry. Volumetry includes methods of analysis where the measurement is one of the amount of space which a certain constituent occupies. Although this may involve evaluations of solids, liquids, or gases, only the latter two are commonly measured automatically. Automatic Orsat-type instruments (*16, 146, 423, 444, 498*) have been used where carbon dioxide is either the desired constituent or a product of combustion of some other constituent, as have apparatus for analyzing small quantities of combustible gases, where volumes before and after combustion are compared. The Orsat apparatus records the shrinkage in volume as the percentage of carbon dioxide by the use of a bell float which mechanically positions a pen arm.

A balloon-barrage purity meter (*169, 170*) developed during the war provided for measurement of oxygen by selective combustion with hydrogen but not with the hydrocarbons also present. The water formed was condensed and the contraction in volume was measured.

Automatic volumetric measurement (*71*) is made of gases in light hydrocarbons which have been liquefied, distilled, and subjected to an adsorption fractionation. The fractions desorbed are identified by thermal conductivity and volumes are measured by a positive and accurate metering device, and recorded by a continuous balance electronic potentiometer. Smith has described in detail a similar apparatus (*480*) where the heat input to the still pot is controlled automatically. The distillate is collected in separate containers and the volumes are recorded.

The volume of solids is measured automatically in cases where the desired constituent is separated by sorption, thus causing a slight increase in the sorbant's volume or configuration caused by volume change. This principle is made use of in hygrometers (*62, 154, 155, 259, 321*) where the sensitive element is a hair or wood fiber or hygroscopic diaphragm whose deformation occurs as a result of a volume change caused by the sorption and which may be used to move a pen for recording purposes. One modification (*383*) of this technique consists of a bimetallic strip of palladium and platinum where the palladium expands as it absorbs hydrogen, thus causing the strip to bend. A recording dilatometer (*265*) has been designed where a mechanical system transfers continuously the accurate readings of an indicating concentric quartz tube dilatometer to a chart.

Another type of volumetric measurement is used in an automatic hardness tester for water (*115*), wherein measured amounts of the water sample and standard soap are added and agitated in a chamber containing a float which seats on the surface of the sample at a height dependent on the volume of foam or suds produced.

Densimetry. Densimetric measurements for determining the densities and specific gravities of systems can be made automatically for gases and liquids (*40, 64, 145, 152, 194, 205, 286, 380, 457*). Smith and others (*476*) published a critical study of eleven instruments available to industry for determining, indicating, or recording the specific gravities of gases.

One method (*397*) involves the principle of imparting a rotating motion to a gas (or liquid) by means of a motor-driven fan. The moving gas in turn strikes the blades on an impulse wheel, producing a torque proportional to the specific gravity of the gas. Other devices (*132, 189*) for giving the specific gravity of gases depend on the effusion principle that the time required for equal volumes of two gases under the same pressure gradient to pass through equal orifices is approximately proportional to their specific gravities. A recently reported German gas density recorder (*189*) consists essentially of a very sensitive balance system made of glass and housed in an enclosed chamber. An enclosed glass sphere is attached to one arm of the balance and an open vessel of the same dimensions to the other arm.

A method (*497*) for liquid density is based on a heavy ball suspended from a sensitive spring and immersed in a liquid. Changes in density are detected by movement of a bar magnet in a coil, and readings are indicated by means of current flow. A bob or a float suspended freely in a flowing liquid or gas has been used (*150*). In one instrument (*52*) the buoyant force exerted on a displacement bulb is measured by a balance enclosed in a gas-type chamber through which the gas flows. The balance deflection is transmitted by a magnetic device to a recorder. A specific gravity indicator (*158*) for heavy hydrocarbons comprises a float submerged in the fluid and attached to a lever which is movable about a fixed fulcrum and is partly counterbalanced by a second float in a pool of mercury.

For measurement of specific gravities of fluids in pipe lines, a recent patent (*151*) provides for two separate metering "rotameter" floats, one so shaped that it is insensitive to variations in specific gravity and viscosity but sensitive to rates of liquid flow, and the other insensitive to viscosity variations but affected by variations in specific gravity and rate of flow. The difference in the positions of the two floats is a measure of specific gravity, providing the flow rate is constant.

The method of balancing two columns of immiscible liquids (*117, 241, 323, 552*) against each other (in a U-tube, for instance) has been made recording for a number of applications. The ammonia content of the outflow gases of an ammonia scrubber is controlled in this manner (*227*).

A recent report on instrumentation and control in the German chemical industry (*189*) describes a suspended vessel type of liquid density meter.

> The liquid flows continuously through a 5-liter vessel suspended on two horizontal springs which are made of tubing to serve as the inlet and outlet connections to the vessel. A change of density of the liquid passing through the vessel causes a change in the load, so that the vessel moves up or down through a distance proportional to the density. A lever system transmits this displacement to a pen recorder.

Recent use of highly penetrating radiating energy or particles such as that from radium for density measurements has been made (see radioactimetry).

Manometry. Manometric measurements are those of pressures and are used with such apparatus as automatic Orsats (*522*) and similar instruments (*226, 279, 309*). A Russian "mechanical gas analyzer for boiler installations" (*562*) is based on the difference in pressure between a column of air and a similar column of flue gas. The balloon-barrage purity meter (*169*) may involve pressure measurements in place of volume decrease (*170*).

For studies of gas production or retention (*554*) by various substances, automatic manometers are frequently used. A recent article (*92*) describes an apparatus for recording evolution of small amounts of gases during long periods. A German recorder for measuring pressure ratios (*189*) consists of a balance system supported on a knife edge. Inverted bells, attached to the arms, are mounted in open top vessels with transformer oil as the sealing liquid. The two pressures to be measured are connected to the underside of the bells, so that the position of the balance beam corresponds to a certain pressure ratio.

Humidity determinations can be made manometrically, based on the different velocities of air with varying water vapor content through porous layers (*191*). Diaphragms, made of various materials whereon are placed mirrors to indicate very small deflections, are used for pressure measurement (*231, 478*). Mirrors generally reflect a small light beam onto a moving photographic strip chart.

The Knudsen gage (*153*) for continuous low pressure measurement operates on the momentum of impact of gas molecules against an aluminum vane. The range is 10^{-3} to 10^{-5} mm. of mercury, although some instruments have been calibrated to 10^{-7}. Safety appliances (*351*) for determining the explosibility of gases spark the mixture and measure the pressure resulting from the explosion by means of a dray needle pressure gage or by depression of a steel diaphragm as indicated by mirrors (*477*).

The well known Pirani gage (*153*), which depends on the thermal conductivity relation with gas pressure in the range of 10^{-2} to 10^{-5} mm., is sensitive to rapid changes in pressure, but is temperature-sensitive and requires calibration for different gases. Thermocouple gages operate on the same principle.

Ionization gages (*153*) are used in the range of 10^{-4} to 10^{-7} and even down to 10^{-10} mm. of mercury absolute. This method is purely electronic in that the gas molecules are ionized by electron bombardment and collect on the plate of a triode. The resulting current is measured as a function of the pressure in the tube.

A control method (*108*) for moisture in paper makes use of a unique operation in the process of steam-heat drying the wet paper sheet as it comes off the paper-making machines. The water of condensation, coming off the wet paper, flows into a container to produce a head which in turn controls a valve admitting steam to the drying rolls.

Viscometry. Measurements of viscosity are finding application in organic analysis more than in inorganic determinations, although the viscosity of water does change with solute concentration. Recording viscometers, frequently under different names, are finding increased use in the control of various processes (*6, 252, 409, 453, 507*)—e.g., the amylograph (*462*) is used in controlling the addition of malt flour to commercially milled flours.

Many automatic viscosity measurements involve the motion of some solid object through the liquid under examination (*543*). A common principle used is based on the torque exerted on propeller blades by the fluid, which can be indicated and recorded. Variations make use of a rotating cylinder, with the measurement being made of the torque acting on a second cylinder or propeller concentrically placed within the first (*59, 68, 261, 370*), the angle of rotation caused by the viscous drag on a stationary bob or paddle suspended from a coiled spring inside the rotating cylinder (*47, 414, 547*), or Wheatstone bridge measurement of the electric resistance of the motor driving the rotating cylinder which depends on the viscosity (*243, 327, 399*). The torque measurements can be made by a strain gage which gives a voltage output indicated by a galvanometer or recorder.

A "viscograph" (*461*) for continuous measurement of the viscosity of starch solutions has a constant-speed stirrer coupled to a synchronous motor mounted at the top of the apparatus. The motor housing is mounted to rotate freely on ball bearings over an angle by which the resistance encountered by the stirrer is read directly. Some instruments provide for rotating the vessels containing the sample rather than the measuring element itself (*51, 159*).

The falling ball type of viscometer has been automatized to the extent of indicating the time of fall electronically (*185, 289, 502*). The moving ball causes a change of inductance in the coils of a tuned circuit, which in turn operates an automatic timer. A commercial instrument (*3*), based on the damping of a swinging pendulum, has been made semiautomatic by placing a mirror on the pendulum which reflects light onto a photographic film or paper.

Gas or liquid viscometers (*105, 132*) dependent on timed flow through a standard orifice have been developed where an electric stop clock is automatically started and stopped. An apparatus (*245*), also based on effusion, continuously indicates the oxygen and carbon dioxide in an atmosphere by passing samples through both sides of a viscosity-effusion bridge, then chemically absorbing the constituent from the sample passing through one side of the bridge without interrupting the continuous flow of gas.

One unique use of flow through an orifice for liquids is made in a commercial model (*47*) for the viscosity of inks used in high-speed presses. The ink sample flows out of a jet in a free steam at an angle 45 degrees from the horizontal. The trajectory height varies with the viscosity, and an indicator is moved up and down along the locus of points formed by the peak of the trajectory at various viscosities.

Recent development work has provided a viscometer similar to the "flowrator" type of volumetric measurement (*149*). The apparatus contains two floats, one above the other. The upper, shaped to be "viscosity-immune," responds only to flow rate, whereas the lower one is "viscosity-sensitive" and is lifted both by increased flow rate and changing viscosity. The difference in the height of the two floats gives an indication of the viscosity, and the values can be recorded by connecting an impedance coil transmitter to each end (*131*).

Thermometry. Methods involving measurement of temperature are classed under thermometry. Such properties as colligative properties—e.g., change of freezing or boiling point or vapor pressure—and dew point are determined by temperature measurement and are classed under this topic. However, the determination of the properties, heat of reaction and thermal conductivity, which may involve measurement of temperature, are discussed under separate sections (calorimetry and thermoconductometry).

Automatic dew point instruments, operating intermittently, have appeared recently (*124, 421, 508*). One type (*113*) acts to condense vapor from the gas sample on a mirror surface whose reflectance of a beam of light is measured by a photocell, which in turn controls the heater circuit and refrigerant application to the mirror. Temperature is recorded by means of a sensitive thermocouple and potentiometer circuit. The condensed liquid may be removed either by a small radio-frequency heater (for quick response) or by a mechanical spring wiper which has the advantage of removing dirt also. A number of dew point recorders of this design for determining atmospheric moisture are available commercially (*8, 10, 174, 199, 328, 344, 474, 509*). For automatic control or determination of the concentration of glycol vapors in air (*176, 188*), a "glycostat" has been devised which also depends on reflection interference due to condensation on a mirror, as measured by a photoelectric cell. Concentration can be regulated within a few thousandths of a milligram per liter by this instrument.

Another type of dew point recorder (*161*) is based on the behavior of hygroscopic lithium chloride in absorbing moisture and dissolving to form a saturated solution. If this is heated to drive off the water, an equilibrium point is reached. When the salt absorbs moisture, current passes between two wires, thereby raising the temperature until equilibrium is obtained. This dew point temperature may be measured by an electric resistance type or thermocouple thermometer. A recent patent (*377*) describes the use of this principle for controlling the concentration of solutions, such as those of lithium chloride or calcium chloride used in dehumidifying air. A boiler containing the sample is heated sufficiently to boil the solution, whatever its concentration may be, and the temperature is then measured and recorded.

Recorders dependent on wet- and dry-bulb temperature measurements (*56, 301*) may consist of two similar electric resistance thermometer bulbs mounted in a tunnel through which a stream of air is drawn by a small fan. One is kept wet by a wick of absorbent cloth.

The dew point method has been applied to the determination of oxygen or hydrogen in inert gases by combustion to water and subsequent measurement of the dew point of the resulting gaseous mixtures. Significant usage of colligative properties in automatic analytical methods has not been described.

Magnetometry. Magnetic properties have been useful for the determination of oxygen by virtue of its strongly paramagnetic nature in contrast to nearly all other gases. Developed during the war both in Germany (*267, 268, 422*) and in this country, the method may use one of several possible actual means of measurement, all of which are essentially magnetic susceptibility determinations.

The Pauling oxygen meter (*33, 391, 392*) depends on measuring the magnitude of the force exerted by an inhomogeneous magnetic field on a dumbbell-shaped torsion balance suspended on a quartz fiber and surrounded by the gas being sampled. A mirror attached to the torsion balance swings with the dumbbell, thus throwing a beam of light onto a ground-glass scale.

Advantage has also been taken of the fact that oxygen's paramagnetism decreases with increasing temperature. A German adaptation (*189, 520*) of this phenomenon depends on the differential heating and cooling of the two halves of a center-tapped platinum resistance coil which form two arms in a recording Wheatstone bridge circuit. Another instrument developed recently in this country, called the Magnotherm oxygen recorder (*139, 215, 426*), is fundamentally similar.

Here a single hot wire is mounted vertically with a strong magnetic field at the bottom, which causes magnetic convection currents that cool the wire in their resultant passage upward. These instruments, which measure the cooling effect on a platinum resistance wire due to the flow of a paramagnetic gas seeking a region of stronger magnetic flux, have the advantage of being more rugged and adaptable to continuous measurement in industry than do those of the torsion balance type.

Magnetic measurements on iron ores (*254*) may be made to determine the iron content, but little automatic use of this has been made. A "carbanalyzer" has also been used for magnetic measurement of carbon in steel. Separation of mixed steel grades in salvage operations is simplified by the use of magnetic analyzers (*493*), which establish differences in chemical composition very quickly.

The measurement of magnetic susceptibility has been extended to the use of an electric field for similar measurements of paraelectric gases such as ammonia and water vapor (*459*). Here also, the cooling effect of a convection current, set up by an inhomogeneous electric field, on the platinum resistance arm of a Wheatstone bridge is the measured phenomenon.

Titrimetry. Automatic titrations are generally carried out by reagent addition from a hypodermic-type syringe buret, where a shaft is rotated to which is attached a piston for forcing out the titrant. Addition of the titrant may be controlled electrically by measurement (*187, 244*) of changes in potential, current, or radiant energy absorption of the solution, or may be made constant while one of the above three variables is measured against time (*28*). Thus far, potentiometric detection of the end point has been the most used (*24, 201, 274, 314, 315, 368, 424, 431, 450, 468, 545, 561*). Robinson's review (*433*) on automatic potentiometric titrations describes the recent developments in this field. The desirable characteristics are: an instrument which both performs the titration and plots the potential versus the volume of the titrant, adaptability to many different electrode systems and plotting of the potential as the ion exponent (pH), adequate mechanical precision and reliability, intermittent titrant addition to allow for lag time, and a rate of reagent addition which is governed by the intensity of buffering action so as to give rapid titration when heavy buffering occurs and vice versa.

An apparatus recently put on the market (*412, 413*) provides two setups whereby one titration may be in progress entirely automatically while the operator is preparing a second solution.

Essentially, all such instruments consist of two parts, the reagent feeding device and the measuring element with its recorder. The feed pump is synchronized with the strip chart, both being driven simultaneously by a relay-operated motor, so that vertical divisions on the chart record the actual quantity of the reagent used. Reagent addition toward the end point is slowed down as the voltage changes per drop increase. Dual electrodes may be used to investigate two solution variables simultaneously (pH and oxidation potential).

Some instruments provide for automatically stopping the addition of titrant when a certain potential is reached. One device (*410*), used for mercaptan (thiol) sulfur in the control of gasoline refining, measures the volume added by means of a motor-driven contact which follows the reagent in the buret, whose movement is communicated to the recording pen by a violin string.

It is advantageous to use an electronic trigger circuit (*369*) to replace an expensive recording potentiometer in controlling the syringe motor. Switching can be made to take place abruptly and definitely at a preset level of input potential, and the apparatus described is suitable for automatic potentiometric and photometric titrations with a high sensitivity.

Automatic determination of hydrogen sulfide and sulfur dioxide (*214*) or ozone (*182*) in gas mixtures can be done titrimetrically after absorption in a predetermined volume of a standard iodine solution until it is decolorized, at which point a photoelectric cell shuts off the gas and admits a standard alkaline solution and an indicator to titrate the acid formed and register the volume of the titrant.

An automatic titration (*464*) which involves electrolytic rather than mechanical or physical addition of the titrant has been recently described.

The apparatus consists essentially of two pairs of electrodes, one called the observing electrode pair, and the other the generating electrode pair, both pairs being immersed in a solution which absorbs the desired constituent from a gaseous sample bubbling through. The potential of the generating pair is controlled by the output side of a direct current amplifier whose input is controlled by the potential difference of the two observing electrodes. Thus a very small excess of the titrant can be maintained by means of negative feedback. When less titrant is detected by the observer electrodes, more current is caused to flow through the generating pair, thus raising the amount of titrant to the required level. Current-recording devices are placed in series with the generator circuit to record the rate at which the titrant is being electrolyzed.

Titration systems in which a constant flow of sample is continuously mixed with varying amounts of titrant which keep the mixture at a predetermined end point have been described. Here the necessary titrant flow rate is measured.

Calorimetry. When temperature increases due to oxidation or chemical reaction of a sample are measured, the measurement process may be called calorimetry. Evaluation of gaseous oxidants or fuels may be made in this manner (*14, 246, 247*). Historically, this was probably the basis of one of the earliest automatic analyses on record. A British patent in 1889 (*407*) described an instrument of two thermometers, one bulb of which was coated with platinum black, and the other was plain. Differences in readings of the two indicated that combustion was going on at the platinum surface and gave means of estimating dangerous concentrations of combustible gases (*38, 348*). Thermocouples were later substituted as the temperature-measuring elements, with recording potentiometers to give continuous readings. A carbon monoxide recorder (*256*) employing this method was developed at the U. S. Bureau of Mines for installation in the Holland Tunnel.

In some cases the process can be controlled so that only one of the combustibles likely to be present will oxidize and the temperature rise can thus be attributed to only that constituent (*195*). The catalyst Hopcalite is commonly used to oxidize carbon monoxide (*148, 351, 353*) to carbon dioxide but not other combustibles, for which platinum black or platinized asbestos is generally used.

Resistance variations of a platinum wire due to temperature changes are also extensively used. Here, Wheatstone bridge circuits in conjunction with recording potentiometers are invariably included (*125, 147, 322, 347, 351, 354, 359, 362*). When oxygen or excess air is the desired constituent, in an atmosphere not containing oxidizable fuels, sufficient amounts of standard fuels are added (*20, 23, 101, 179, 322, 363, 494, 495*) to consume all the oxygen present, thus giving a measure of the concentration according to the heat evolved. Many commercial instruments, some of them portable, based on both principles of temperature measurement for use as safety devices in mines, fuel tanks, and tunnels, are on the market (*20, 58, 70, 319*). An elaborate "multipoint" combustible gas alarm system (*120*) continuously records at one location concentrations of gases or vapors at six to twelve remote sampling points.

Curvature of a bimetallic strip (*1, 529*) has also been used as the means of temperature measurement, but not extensively for automatic analysis. Another design (*500*), in which expansion plays a part, makes use of a plurality of low-creep wires covered with catalyst and carrying an electric heating current. When gases surrounding the wire oxidize, the resulting elongation of the wire causes a movable resilient fitting to operate an external device.

Catalytic poisoning provides another variation of basically the same type of measurement (*48, 82, 401*). For example, use is made of the fact that traces of gases poisonous to humans also destroy or inhibit the catalytic action of platinum in the oxidation of methanol vapor. The apparatus consists of finely divided platinum-covered wire continuously catalyzing the oxidation of the alcohol vapor to maintain a temperature at which the bridge is in equilibrium. When the catalyst is poisoned (by carbon monoxide, or hydrogen cyanide, among others) oxidation stops or slows down with resultant temperature drop and bridge unbalance.

A Russian calorimetric method, which does not involve oxidation, depends on the effect of $ZnO.Cr_2O_3$ in catalyzing the recombination of atomic hydrogen in hydrogen molecules (*272*). The method thus measures the concentration of hydrogen atoms in a hydrogen flame thermoelectrically.

Chronometry. When time is actually the measured variable in chemical reactions, the method may be called chronometry. As was mentioned in the section on titrimetry, whenever the titrant is added at a constant rate, time instead of volume is conveniently measured, and may be directly proportional to the percentage of the desired constituent as long as other influencing factors are held constant.

Another automatic method recently published in Russia (*518, 519*) is the measurement of the length of time required for the complete electrolysis by a mercury cathode of metals out of a solution at constant current. In this case the recorded qualitative variable is the potential. A steplike graph is obtained in which the number of minutes between the points of standard potential, marking the end of the separation of one element and the beginning of another, is a measure of the quantity of metal deposited. This is true, of course, only if the amperage is maintained constant, which is done electronically in the instrument described, and the current efficiency remains 100%.

As an example of a titrimetric procedure specially designed for chronometric measurement (*27*), a method of chronometric spectral analysis may be cited where the measurement is of the time a band of radiant energy takes to disappear from the spectrum when a constant-speed reagent is added to the sample. Time may actually be the measured quantity in the study of properties other than chemical reactions. An example is the method for viscosity where the time required for flow through a restricted opening is measured.

Sonimetry. Recent applications of the measurement of absorption of waves in the sound regions have been made for several types of gas analysis (*73, 308, 530*). Equations have been derived (*242*) showing that, in cases where the single components of a gas mixture have different ratios of specific heats, the velocity of sound through the mixture depends on the single values for sound velocity through the components. Practical applications are, of course, limited to essentially binary systems, and nitrogen-hydrogen, air–carbon dioxide, and hydrogen–carbon dioxide systems using interferometric techniques have been studied (*281, 491, 542*). The same idea has served for molecular weight determinations of liquid polymers.

One acoustic gas analyzer (*176*) which has been commercialized consists of an acoustic resonator with frequency a function of molecular weight and therefore the gas concentrations, provided the two components are of different molecular weights. A similar apparatus has been used for continuous density measurements of gases in limited cases (*346*). A recently described electronic device (*110*) based on the velocity of sound in gas mixtures gives satisfactory continuous readings of the compositions of the sample passing through it.

High speed of response and pressure insensitiveness make the sonimetric method (*344*) a useful way of controlling high-velocity gas blending. Another advantage is that the system is capable of operating under extremely corrosive conditions, for minor changes of dimensions due to corrosion or build-up have negligible effect on the operation of the cell.

Emissimetry. The fact that chemical elements, suitably excited, emit radiant energy has led to the analytical method which may be called emissimetry. Gases, liquids, or solids may exhibit this phenomenon, and the means of excitation may be thermal or electrical. The wave lengths produced depend on the type of excitation used, as well as the sample itself and other conditions, as do also the band widths, which vary from narrow line spectra to the wider bands associated with fluorescence and phosphorescence.

Routine operations in conducting emissimetric (emission spectrometric) analyses on solid samples, usually metal alloys, have been widely investigated for their automatic possibilities within the past decade. The specific operations of presparking, shutter opening and closing, cutting off the spark, shifting the plate, and changing the sample by motor-driven turntables, have been successfully automatized (*178, 193, 258, 563*). Automatic measurement of the line intensities, or their ratios, has been effected by introducing the use of electron-multiplier phototubes or Geiger-Müller photoelectron counters. Densitometers (*527*) are also on the market for automatically recording line densities on photographic plates by means of photocell response to light transmission through the plate and its emulsion.

Spectacular instruments including many of the above operations are being manufactured (*12, 22, 42, 50, 66, 204, 212, 262, 451, 452, 523, 536*). They cut the time required for an emissimetric analysis to a matter of minutes or even seconds, thus surpassing the wildest dream of the analyst.

> Applications have been described for steel, aluminum, and magnesium alloys, where up to eleven elements may be determined simultaneously. All the operator needs to do is to prepare the sample for use as one of the electrodes, insert it in the holder, and then read the results directly in percentages from dials. The principle involves placement of photomultiplier tubes behind slits, placed where the photographic plate would ordinarily be located, at positions corresponding to the wave length of the line whose intensity is to be measured. The photocurrents thus produced are stored in suitable condensers which discharge at certain points to actuate the counters.

One automatic use of spectra produced by thermal or flame excitation has been a leak detector (*312*) used for methyl bromide, Freons, and other noncombustible gases. The sample comes in a suction tube, and a rapid flow of acetylene draws it into the flame where heat decomposes it into free acids which give a change of color to the flame when it hits a copper plate. Flame photometers have seen increasing use in the past few years. Another automatic method utilizing radiant energy emission from a gas or vapor has been developed for determining concentrations of lead in air using spark excitation.

An emissimetric method where the source of radiant energy is not the sample (*395*) employs a palladized platinum wire at 200° C. contained in a tube through which air is passed. The presence of methane or ethane causes the wire to glow, and its intensity as measured by a photoelectric cell is in certain limits proportional to the concentration.

Transmittance.. The process complementary to emission of radiant energy is transmittance, and it is in this field that perhaps the largest number of applications of automatic methods exists. Tremendous advantages inherent in radiant energy transmittance measurements make them especially adaptable for continuous automatic analyses. Of particular importance are the facts that the sample being tested is usually undamaged in the methods used and that the photoelectric or thermal response to changes in transmittance (and thus concentration) is practically instantaneous. From the standpoint of automatization, the simplest cases, of course, are those where the desired constituent itself absorbs radiant energy of a particular wave-length band where there is negligible interfering absorption by other substances present. If the magnitude of the transmittance (-cy) is not specific to the unknown in question, either a color (or absorption band) must be developed which bears a direct relationship to the unknown in question, or the absorption interference of the disturbing ingredients must be removed. The latter may be accomplished by removing the interfering constituent itself, by changing the chemical or physical state of the impurity so as to decolorize it, or (in cases where the constitutents have fairly constant concentrations and are present in small amounts or have absorption bands which merely overlap that of the desired constituent and do not coincide exactly) compensating for the error by placing an equivalent amount of the impurity in the second (100%) beam of a double-beam instrument, or in the reference "zero" sample of a single-beam instrument.

The various automatic methods are considered below under the subtopics: absorptiometry, thermoconductometry, turbidimetry, refractometry, and diffractometry.

ABSORPTIOMETRY. The absorption of incident radiant energy of four wave-length regions—viz., infrared, visible, ultraviolet, and x-ray—by substances has long been used as a basis for measurement of concentrations. For many years comparisons between standard and unknown solutions had to be made by eye. The subsequent development of efficient photoelectric cells and means of measuring their small currents has provided the accuracy and reproducibility needed to permit a phenomenal growth in the use of quantitative absorptiometric measurements and the automatization of many of these (*167*).

Significant advances have been made in recent years for analysis of fluids in a continuous manner, particularly in the use of infrared and ultraviolet absorption equipment. Usually in the simplest cases, the sample fluid (either a liquid or gas) is passed through appropriate cells in the absorption-measuring instrument, where radiant energy–sensitive cells measure the proportion of radiant energy absorbed at more-or-less specific wave lengths. The fundamental principles of measurement and construction of apparatus do not vary essentially with the wave-length region used. There must invariably be a radiant energy source, the sample, a means of dispersing the radiant energy, and means of measuring the radiant energy intensity.

Main consideration here is given to the devices that provide for automatic sampling as well as measurement, although much work has been done on automatizing the measurement of the absorption spectra of single samples (*114*).

Absorptiometry of Gases. Various procedures have been devised in cases where absorptive capacity in the desired wave-length range must be developed (*560*). In general, such automatic operations for gases consist of one of the following: conversion to a different gas by means of a chemical reaction or by altering the physical conditions; mixing the gas with a solution to develop an absorption band directly related to the concentration of the desired constituent; or reaction of the gas with a solid reagent which is coated on a moving strip of cellophane. The cases where the analysis is of a gas which is already dissolved in a liquid are considered under liquid samples.

Many automatic apparatus are in use for measuring the radiant energy passing through the sample containing the gas or vapor in question (*103, 419, 484*). Infrared measurements (*253, 256, 406, 556*) are in common use for analysis of organic systems. The wartime development of infrared analyzers (*22, 168, 337, 375, 557, 558, 559*) for butadiene and styrene plant streams is an excellent example.

These may be double-beam systems employing a single source of radiant energy, a split lens or mirror, a sample cell, and various filters through which one or both beams may pass, and two electrically opposed detectors upon which the beams are focused. Selectivity of wave-length band is provided by the filters and by detectors having selectively absorbing radiant energy receivers. A vacuum-tube amplifier, working on the bolometer (or thermocouple or "radiometer") output, actuates a potentiometer-recorder which automatically restores bridge balance along with recording the analysis of the sample. Automatic standardization at regular intervals may be provided by the introduction of a standard into the sample cell by automatic valves which cut off the flow of the sample and admit a standard gas.

An infrared absorption meter (*189*) developed in Germany in 1938 actually measures the radiant energy absorbed and not that transmitted.

Two radiating sources, connected in series, are heated to a red heat, one transmitting into the analysis cell and the other into a standard cell. The pressure difference, which results from a difference in the radiant energy absorption between the two cells, causes a deflection of a membrane separating them, resulting in a change in the capacity of the condenser of which it forms one plate. The voltage oscillations arising from these periodic pressure changes are amplified, rectified, and then recorded.

The relative humidity of air has been determined by comparing the relative spectra of air with standard samples in the infrared region (*528*). A "spectrographic hygrometer" measures the total amount of water in a vertical column in the atmosphere (*160*). In this case the sun is the source.

Several applications of infrared-acoustic analysis make use of a microphone detector (*81*). The microphone responds to differences in the radiant energy coming from the reference and analysis chambers, and its output is amplified, rectified, and applied to a millivoltmeter recorder. A more recently developed instrument (*532*) of the same type has been described as instantaneous in response and determines 0.1 mg. of ether in 1 liter of air.

Photometric methods using ordinary light sources have been developed for continuous recording of concentrations of carbon disulfide and hydrogen sulfide in the viscose rayon industry. Methods for nitrogen oxides (*285, 331, 388*) are available where nitric oxide is converted to nitrogen dioxide for analysis in processes such as nitric acid production.

Various automatic methods are in use where the gas is passed through a solution whose transmittance depends on an absorption band developed by reaction of the desired constituent with the solution used (*74*). Examples include oxygen with alkaline pyrogallol (*310*), hydrogen cyanide with iodine solution (*531*), nitric oxide with Griess solution (*445*), hydrogen sulfide with ammonium molybdate solution (*483*), and carbon dioxide–forming compounds with various color indicators (*69*).

A British patent (*106*) describes a device for the "photoelectric recording of variations of chemical composition of a substance." The substance (gas or liquid) is brought into contact with a moving piece of transparent film impregnated with a substance which reacts with the desired constituent to alter the intensity of a light beam.

Various gases and vapors have absorption bands in the ultraviolet region. Some instruments have been described as applicable to any of these, whereas others are made specifically for a particular substance (*206, 524*). Mercury-vapor lamps are frequently used as sources in devices for measuring mercury concentration itself (*172, 374, 469, 553*). The National Bureau of Standards has developed a ground-based instrument (*84*) for total ozone in the stratosphere.

The well-known Beckman quartz spectrophotometer is available in a recording model (*32, 440*) for continuous automatic analysis of process streams for substances which absorb in the ultraviolet region. The radiant energy transmitted through the sample cell strikes a photocell, setting up a current which flows through a load resistor to produce a voltage which is amplified and recorded. Because changes in the source output, phototube sensitivity, and dirt accumulations on the windows of the cell will affect the readings, an automatic standardization mechanism is provided to restandardize the instrument periodically.

Absorptiometry of Liquids. A number of methods are based on the measurement of a substance occurring in a liquid sample by transmission of radiant energy through the solution itself (*429*).

A 1906 German patent (*488*) entitled "automatic method of chemical analysis" involves passage of light through the substance onto selenium, and the intensity depends on the concentrations of various constituents present.

Automatic control of dyeing baths has been made possible by use of photoelectric devices (*85, 116, 263, 429*). A versatile commercial "continuous-flow colorimeter" (*404, 438*) gives immediate and direct indication of the light transmittance of a liquid flowing through the instrument. A recent patent describes an automatic water softener which uses a photoelectric cell for checking water hardness automatically and controlling the softening process (*428*). For the continuous determination of the bivalent copper present in the ammoniacal scrubbers used to remove carbon monoxide in the TVA ammonia plant, the solution to be tested flows through the annular space between two concentric tubes in an all-glass, light absorption cell surrounded by a light source operating on a stabilized voltage (*61*). An infrared device has been described which can easily be converted into an automatic control device in the production of natural and synthetic rubbers (*26*).

Two devices for measuring continuously the oxygen saturation of circulating human blood have been developed where the measurements are made in situ by means of bichromatic photoelectric colorimetry (*210, 349*). The sampling and measurement point is generally the ear, and the entire optical system, consisting of a miniature lamp bulb, two color filters, and two selenium barrier-layer photocells, weighs only 30 grams and slips over the shell of the ear.

Numerous devices are in use where chemical treatment of the sample is necessary to obtain a suitable absorption band. A British patent (*329*) describes an apparatus for testing water where the sample and reagent are mixed automatically and then conducted through an observation chamber in which the coloration (or turbidity) of the liquid can be measured. Several colorimetric autoanalyzers for pH have been described (*53, 67*). In one (*67*), thymol blue indicator drops continuously into the flowing solution and the transmitted radiant energy passes through a yellow filter before reaching the photoelectric cell.

Colorimetric determination and automatic recording of the metal contents of aqueous solutions (especially calcium and magnesium in hard waters) are done by adding an alkaline solution of a dye to the sample, so that a chemical reaction with the metals produces a decreased or increased absorption to an extent that corresponds with the metals content (*280*). Iron is determined with thiocyanate in a similar apparatus. The "hydrotimeter" (*443*) measures water hardness by the amount of light transmitted by the foam formed by a measured volume of the water sample with a standard soap solution. A continuous sugar analyzer (*475*) combines the sample automatically with a dilute solution of 1-naphthol in concentrated sulfuric acid.

Gases in liquids are also measured automatically by mixing with various reagents. Oxygen can be continuously determined by means of a potassium hydroxide and pyrogallol mixture (*521*). Chlorine in water is determined using the *o*-tolidine reaction in the alkaline (yellow) range (*72, 225*).

X-ray absorptiometric measurements are particularly applicable to elemental analysis of organic compounds. One instrument (*86, 293, 381*) uses a "white" x-ray source, an adjustable slit, a rock salt monochrometer, a 1- to 5-mm. cell containing a liquid sample. A Geiger counter gives measurements virtually independent of the physical or chemical state of the materials (*171*).

Typical applications include the determination of tetraethyllead in gasoline, acid in water, sulfur in oil, ash in coal, and chlorine in a plastic. This model consists of a tungsten target tube source of x-rays (0.35 to 1.07 A.), a fluorescent screen (*176*) to produce visible radiant energy which is then measured by a multiplier phototube, an amplifier, and an indicating instrument. The x-ray beam is interrupted by a synchronous motor-driven chopper so that the beam passes alternately through the reference and sample cells. The automatic version of the instrument gives suitable records of continuous processes.

THERMOCONDUCTOMETRY. All gases have the ability in varying degrees to conduct or transmit heat waves (infrared radiant energy) from a hot source to a cooler location, and this property has been used extensively for many years in the continuous determination of various gases (*147, 341, 356*), particularly carbon dioxide and hydrogen. Advantages include nearly complete independence of varying pressure, ambient temperature and flow rate, high sensitivity in many cases, robust construction of apparatus, small time lag, and low cost of operation (*540, 541*). Like many other physical measurements, however, it is systemic in nature and gives no qualitative information regarding the chemical nature of the constituents present, so that the chief field for the method is in binary mixtures of known constituents (*122*).

The principle usually involves a Wheatstone bridge measurement (*482*) of the variation in resistance of a platinum or tungsten wire, which constitutes one arm, due to temperature changes brought about by the changing heat conducting ability of the gas passing through the cell that surrounds the wire. This cell may be constructed along one of three general lines (*142*): (1) a simple by-pass system where a standard gas is sealed in one side of the bridge while the sample passes over the platinum spiral in the other side (used for determining hydrogen in oxygen or nitrogen, carbon dioxide in flue gas, sulfur dioxide in burner gases) (*17, 75, 119, 123, 142, 186, 203, 294, 295, 300, 332, 439, 465*); (2) a double by-pass cell which is the same except that gases flow through both sides of the bridge (useful for comparison of a gas before and after a chemical reaction or absorption, as in the determination of carbon monoxide, various combustibles, ammonia, acetone, or methanol) (*75, 142, 294, 396*); and (3) an all-quartz or glass cell for corrosive gases where the platinum spirals are quartz or glass protected, and the tubes are similarly lined (for sulfur dioxide, hydrogen chloride, chlorine, or hydrogen sulfide) (*75, 294*).

A method for the determination of argon in ammonia-synthesis gas, where the thermoconductivity is measured, has been described (*61*). The gas is first freed of ammonia by scrubbing, and from hydrogen and methane by oxidation with a cupric oxide–ferric oxide mixture. The oxidation products are then removed, and the purified mixture of argon and nitrogen is passed through the thermoconductivity instrument which records the argon content continuously.

Many organic vapors can be measured by this principle (*75, 173*), providing the impurity has a different thermal conductivity from that of the gas being analyzed or can be changed by chemical reaction on a known qualitative basis to a useful one.

A patent (*396*) for a method of analyzing gas mixtures with oxygen and one or more other gases, such as carbon monoxide, carbon dioxide, or methane, describes a device for adding hydrogen (or an equivalent combustible gas) to the mixture, a combustion chamber no part of which is catalytic to the reaction of the oxygen or hydrogen with any of the other gases, and means for measuring the thermal conductivity of the mixture after the combustion. A similar method (*75*) for recording oxygen, carbon dioxide, carbon monoxide, and hydrogen in a sample of combustion products, involves direct comparison of thermal conductivity of the sample with that of a reference gas or comparison of thermal conductivity before and after chemical absorption and combustion. A single recorder is used by means of an automatic timer to connect it to each analysis cell in succession, so as to give four differently colored lines representing the results of all four constituents on one chart. For determining dissolved oxygen in water, the sample is continuously scrubbed by hydrogen to displace the oxygen that is forced out and then measured, in this case by combustion to carbon dioxide over a heated carbon rod (*75*).

Measurement of thermoconductivity in a magnetic field (*215, 266, 422, 426, 520*) is described in the section on magnetometry.

TURBIDIMETRY. Turbidimetric measurements are used mainly in smoke density recorders, although there are other applications. Several commercial versions of smoke density indicators and recorders are available (*19, 302, 402, 544*). In general, the radiant energy source is a voltage-controlled lamp (in one case, a sealed-beam spotlight) housed in a dust-tight casing mounted in the duct or passage through which the smoke passes, while the detectors are thermopile or bolometer units which pick up the radiant energy filtering through the smoke. In one design (*302*), a set of five lamps, each a different color representing five progressive density ranges, is the indicating component. Sampling from two points is frequently resorted to, to avoid stratification effects.

An apparatus for determining carbon disulfide (*133*) passes the sample through a furnace where sulfur dioxide is formed. This then passes through a water bath, and the fog produced goes into a fog chamber where its effect on the transmission of a light beam is measured continuously. For the continuous determination of sulfur in a fluid, the stream is brought into contact with mercury (*249*). This fluid is then separated from the mercury and the mercuric sulfide precipitate and its radiant energy transmittancy is measured.

REFRACTOMETRY. Refractive index values as indications of changing composition of liquid systems have recently been automatized. One type, originally developed for butadiene purity measurement, gives continuous visual indications instead of indicating or recording automatically (*467*). A dipping prism is mounted in a pressure cell through which the liquid flows continuously at process pressure and constant temperature.

An instrument (*93*) designed in Sweden as part of a "self-registering apparatus for adsorption analysis" conducts the solution (having passed through suitable adsorption columns) into one of the halves of a double prism (the other half filled with the solvent only).

> The light beam passes through both prisms and thence through a hexagonal prism which divides it into two parts, which then illuminate two photocells. When the sample has a refractive index different from the solvent, the beam will deflect from its normal straight path and thus illuminate one photocell more than the other. Amounts of sample are measured gravimetrically rather than volumetrically by means of a spring-type balance. The recording devices for refractive index and weight are coordinated with crossed mirrors, and the change in refractive index is recorded as a function of weight. The instrument is capable of wide ranges of sensitivity and capacity, and is applicable wherever continuous observation of changes in concentration is important, as in distillation, titration, and extraction.

A refractometer (*25*) for continuously analyzing a fluid stream has the sample flow through a hollow prism, passes radiant energy through it, and causes the spectrum produced to impinge through a wide slit on a photocell.

> A change in refractive index results in a shift of the spectrum produced, which in turn causes a corresponding amount of it to be cut off, so that the amount of radiant energy striking the photocell varies. This method includes improvements for speeding up the change of photocell current with change of refractive index and for cutting the effect of changing radiant energy transmittancy by the fluid. Instead of collimating a beam from an ordinary source, a very narrow luminous line—e.g., the mercury green line—of substantially monochromatic radiant energy is collimated and passed through the prism, and then impinged on a plane containing at least one sharply defined edge which may be a slit or a sharp narrow zone of insensitivity on a photocell. A very slight movement of this straight line will cause an abrupt change of the dark cell current of the photosensitive device. The apparatus was designed to permit control within very close limits on solutions of dicyandiamide in liquid ammonia for use in the production of melamine, and the reaction for producing acrylonitrile by the interaction of hydrocyanic acid and ethylene oxide.

A photoelectric refractometer (*255*) which is somewhat differently constructed is based on the change of the intensity of light instead of the measurement of the angle of total reflection (as in classical methods).

> In this method the responsive light beam does not pass through the substance under test, so color and transmittance do not interfere. The apparatus consists of a round glass rod bent into U-form immersed in a liquid contained in a black compartment. In air considerable light is transmitted along the rod by multiple internal reflection, but in a liquid, more light will escape at the bend and less will be transmitted internally to the photocell at the other end. The refractive index of the glass rod sets the upper limit of that which can be measured by this method. A better arrangement is a comparison system where two rods and two solutions (one a standard) are used together with two photocells set in a null circuit for a maximum sensitivity of two units in the seventh decimal, while the workable accuracy is about two in the fifth place.

DIFFRACTOMETRY. A number of commercial x-ray spectrometer instruments are available for many problems, especially where crystalline structure is concerned (*78, 177, 373, 381, 418, 479, 548*). Automatization has been achieved to the extent of incorporating a traveling Geiger counter for recording the intensity of the diffraction rings versus the spacing of them. However, the technique has not been adapted to the measurement of continuously changing samples. Manual preparation of powdered samples is required.

Reflectometry. Reflectometry involves the measurement of radiant energy waves reflected by opaque surfaces of solid objects. Strips of paper are impregnated with a reagent which reacts with the desired constituent to produce a color dependent on the amount present, and they have been used as the basis of continuous analyses. Most instruments using this principle involve lead acetate paper for the determination of hydrogen sulfide (*34, 96, 133, 504*). A recently commercialized hydrogen sulfide analyzer (*446*) brings the sample to a constant predetermined temperature and humidity, then meters the flow of gas through a restricted area of permeable white reactive tape impregnated with lead acetate and other reagents to assure stoichiometric conversion to brown lead sulfide of all the hydrogen sulfide. An arrangement used for the determination of mercury (*172*) consists of a truncated cone which acts as an air chimney, providing a reasonably constant air flow by the heat of a light bulb. The measurement here is made visually by means of comparison with a calibrated color chart, and it is based on the principle that the selenium in selenium disulfide is displaced by mercury, producing black mercuric sulfide.

Reflectance by a plane surface is also the basis of the dew point instruments mentioned above (see section on thermometry), where condensation of vapor on a mirror is detected photronically, and the temperature at which this occurs is measured.

Nephelometry includes measurements, dependent on the well-known Tyndall effect, of samples similar to those used in turbidimetric methods but involving measurement of the radiant energy scattered or reflected to the side by the particles in the solution rather than that which is transmitted through it. One device (*196*) was designed for measurement of the light dispersed at exactly right angles to minimize the background lighting interference. It has been used for comparing the concentrations of very dilute aerosols, but is applicable to a wide range of uses in colloid chemistry.

A nephelometer (*248*) for rapid testing of penicillin growth and similar preparations consists of a multiple carrier, maintained in a constant temperature bath, which revolves into position for instant measurement of bacterial growth as indicated by the Tyndall effect in any particular tube. Comparisons are made of cultures containing penicillin with one not containing it (as a control), and these give the effect of penicillin on the growth as shown on a graph recording the effect of numerous penicillin preparations on rapidly growing cultures with respect to time, a measure of the potency of the penicillin under test.

Conductometry. Electrical conductivity has long been recognized as an adequate quantitative indication of concentration. In theory, a conductometric cell is a mechanical device for isolating a column of solution so that the cross-sectional area and length are constant and there is no contact resistance at the electrodes. Desired properties of the electrodes, which are particularly important in continuous conductometric measurements (*257*), include: good conductivity of electricity, resistance to corrosion, resistance to erosion, strength to stand abuse, and a surface structure tending to cut polarization error and adsorption. Industrial applications do not require the refinements necessary in research work, because reproducibility, not accuracy, is the vital specification.

Measurements on solutions and gases, in contrast to those on solids (due to the sampling problem), are readily made automatic. However, examples of measurements in all three physical states, including those where the sampling operation is largely manual, are considered here.

CONDUCTOMETRY OF SOLIDS. Solids are generally measured conductometrically for the purpose of estimating their water content. Several setups are available for use in paper mills, where the amount of water present in the paper sheet as it comes off the rolls in the drying section is important. In one (*112*), the current flows from a detector roll through the paper sheet to the machine (or idler) roll, which is grounded. Care must be taken to ascertain the effects of pH, speed, static, temperature, fillers, and dyes. Sometimes the multiple detector-roll system is preferred, because it consists of a number of detector rolls mounted side by side across the paper to indicate the moisture content across the sheet. Moisture in textiles (*492*) can be similarly measured.

Conductometric moisture measurements are made on other solids (*449, 512*) such as straw, grain, cottonseed, meat, cheese, crackers, sand, soil, lumber, clay, and clay products. One portable "moistmeter" (*127*), designed for moisture in sand, has the conductivity cell mounted on the end of a long handle so that the operator merely pushes the end into the pile of sand and reads the dial. A slightly elaborated apparatus (*234*), used for moisture in soils, consists essentially of a block of plaster of Paris which is permanently embedded in the field soil. The resistance of this block is a function of its water content, which is in turn closely related to the amount of moisture in the soil surrounding it.

A number of methods for relative humidity of air depend on measurement of the conductivity changes of solids which are dependent on the amount of water vapor in the surrounding air. One very simple one (*63*) consists of a dry salt-impregnated cotton wick, whose conductivity is measured by two platinum electrodes attached to it. Another type (*511*) is based on the electrical resistance of a surface when dew is deposited on it. Recently, more elaborate devices (*107, 135, 136, 143, 260*) have been patented and commercialized. A typical one (*7*) uses hygroscopic film—e.g., a polystyrene resin on an aluminum base.

Measurements on solids for constituents other than water have also been made (*233*). Sizing in paper is estimated (*54*) continuously by measuring the change in resistance as moisture permeates the sheet. A rapid method for the analysis of commercial aluminum alloys and magnesium alloys of various types by electrical resistivity gave a straight-line relationship for correlating the magnesium and aluminum content (*442*). Similar studies show that the resistivity of certain types of silicon-iron sheets is practically a linear function of the silicon content (*18*).

CONDUCTOMETRY OF LIQUIDS. Most conductometric methods involve measurements on liquids, particularly aqueous systems (*231, 236, 237, 408, 486, 487*). Applications include determination of purity of distilled water (*299, 303*), chlorine concentration of tap water, examination of boiler feed water and condensates (*219, 238, 313*), uniformity of mineral waters (*235*), and concentrations of pure solutions (*36, 141, 338*). A conductometric indicator (*46*) for nitric acid in the production of concentrated nitric acid has the purpose of signaling the presence of nitric acid in the spent sulfuric acid pumped from the acid concentration tower.

A very sensitive device (*43, 44*) for indicating small changes in electrolytic resistance depends on variations in the Q of a vacuum tube oscillator caused by variations of a resistance capacitatively coupled thereto. A special device has been designed for measuring conductivity at high temperatures with continuous readings (*372*).

Control of boiling in sugar extraction (*264*) has been done conductometrically when other factors such as salt content and quantity of sugar already crystallized do not greatly interfere. Plant control (*299, 303, 379*) of cleaner baths, acid or alkaline rinse waters, and dip baths has been safely accomplished conductometrically, as has also a safety device in war plant acid coolers (*90, 360*). In a method for chlorine in water (*535*) a built-in synchronous motor-driven buffer pump feeds a controlled amount of acetate buffer into the sample water to maintain its pH essentially constant. For the determination of dissolved oxygen (*386*) the sample is passed through the cell to which a water-soluble electrolyte is added.

Conductivity recorders are used in the sea water bromine process (*209*) for measurement of water salinity to indicate and record parts per million of bromine in a range of 35 to 70 p.p.m.

Automatic temperature compensation can be effected in two ways. One involves simply the use of a reference conductivity solution having the value of conductance which is required at the control point. Changes in temperature then affect both cells simultaneously and cancel out in the bridge network. The second method consists of the use of a mechanically balanced temperature recorder which measures continuously the temperature of the solution and resets the temperature-compensating slide-wire in the conductivity instrument bridge network as changes in the temperature of the process material occur.

CONDUCTOMETRY OF GASES. Ionizable gases can be determined by passing samples through liquids whose conductivity changes when the gas being measured reacts with or dissolves in the solution located between the two electrodes of a conductivity cell (*61, 129, 163, 384, 385*).

A typical apparatus (*162, 333*) designed for detection of war gases is so arranged that disturbance of the bridge balance by alteration of the conductivity of the liquid between the electrodes causes one of two lamps to light, one for clear, the other for danger. Gases and vapors which are similarly determined include carbon monoxide (*61*), carbon dioxide (*61, 129*), carbon disulfide, hydrogen sulfide, nitrogen oxides, phosgene, lewisite, phosphorus trichloride, chlorine, sulfur dioxide (*181, 525*), and ammonia. Some un-ionizable samples require oxidation to a form which will ionize readily (*61, 121, 163, 213*). Samples containing chlorine are passed through a dilute solution of hydrochloric acid where most of the chlorine changes quickly into hydrochloric acid and hypochlorous acid, which increase the electrical conductivity (*287*). Ammonia-containing gases are bubbled into a conductivity cell through which dilute sulfuric acid is flowing at a constant rate (*61*).

The determination of sulfur dioxide in air is accomplished in the Thomas sulfur dioxide autometer (*505, 506*), by absorbing the sulfur dioxide in a simple countercurrent absorber. Variations and improvements on the basic apparatus have been developed to make it more adaptable. Total volatile sulfur-compound content can be determined, as can organic chlorine compounds, such as chloroform, carbon tetrachloride, ethylene chloride, and chlorobenzene. A portable unit employing this principle has recently been developed and is available commercially.

A method for determining and controlling the ratio of oxygen and combustible gas supplied to furnace burners involves measurement of the electrical conductivity of a flame produced by the oxygen and gas mixture (*77*).

Several devices involving conduction from filaments, as in vacuum tubes, have been announced. One depends on thermionic emission of a tungsten filament in a vacuum which drops when oxygen enters (*176*). Another (*546*) depends on the fact that platinum, heated to incandescence, freely emits positive ions in air (not electrons as in tubes) and does not readily oxidize. This emission increases in the presence of halogen vapor compounds, of which Freon and carbon tetrachloride are good examples. Air is forced at a constant low velocity between two closely spaced cylinders, the inner one kept red hot and the outer one kept at a negative potential. The sensitivity varies with the velocity of air flow between cylinders. Thus, at a rate of 1 inch per second it may be 1 p.p.m.

Potentiometry. The most commonly measured chemical variable based on measurement of electrical potential is hydrogen ion concentration (or activity) or pH (in fact, the "p" is derived from the word potential). In many industries processes cannot be carried out without pH control. In others it may minimize corrosion, an especially important factor in boiler feed water where the prevention of corrosion, embrittlement, and scale formation is vital. In general, the various methods involve measurement of the electrical potential produced by a special cell in contact with the sample as compared with some type of standard reference cell.

Early recording methods based on hydrogen or oxygen electrodes were unsatisfactory, and the glass electrode has since come to the fore. Various glass electrode apparatus have been described using alternating current supplies (with various devices to eliminate voltage fluctuations), thus being suitable for lengthy continuous recording (*76, 200, 276, 283, 296, 311, 389, 420, 466*).

Several companies manufacture continuously indicating or recording instruments of the glass electrode type (*57, 75, 89, 239, 297, 298, 304, 350, 376, 403*). Some are line-operated (115-volt alternating current), may have automatic temperature compensation, and can be provided with a multiple-pen recorder for giving the pH at various points in a continuous process.

Applications of pH control are important in the following chemical unit operations: precipitating, coagulating, neutralizing, electrodepositing, fermenting, bleaching, settling, hydrolyzing, polymerizing, sizing, and dyeing. Specific industries such as paper (*80*), synthetic rubber, sugar, ore flotation, food, textiles, petroleum (*400*), and penicillin and other bacteriologicals have made extensive use of automatic pH determination and control. The pH of process and municipal water supplies, cooling water, and boiler feed water is also commonly controlled automatically.

The apparatus generally consists of the electrode assembly, amplifier unit, and a recording or indicating potentiometer. The electrode assembly may be of either the immersion or flow type. Requirements of good indicating electrodes include reliability over wide temperature ranges, and protection from stray currents. The requirements for a good reference electrode or half-cell are: constancy of potential regardless of temperature changes, constancy of potential over long periods of time, ease of maintenance, freedom from poisoning by solution in which it is immersed, and freedom from pH effects. The calomel half-cell meets these requirements for a standard cell and is most commonly used.

Compensation for the changes in the resistance of glass resulting from changes in temperature may be accomplished by incorporating a resistance thermometer with the electrode assembly so tied into the electronic measuring circuit that continuous and accurate compensation is effected.

Use of bismuth-calomel and antimony-calomel electrodes for automatic pH measurement has been described (*37, 342*).

Constituents other than hydrogen ions have been measured potentiometrically. A method for oxygen in gases has a detector cell consisting of a hollow carbon electrode immersed in an electrolyte in a plastic container (*355*). The hydrogen evolved at the carbon electrode causes polarization, which results in a decrease of voltage and current. Oxygen from the sample diffuses through the wall of the electrode and combines with the hydrogen, thus depolarizing and raising the current flow. An instrument for continuous alveolar carbon dioxide bubbles the gas through a solution of sodium hydrogen carbonate and measures the potential changes produced (*538*).

Potentiometric measurements in the sea water bromine process (*83*) include pH, oxidation potentials, gas analysis, sea water salinity, and temperature. Controllers operate with pneumatic control systems, so that the production of liquid bromine from raw sea water is almost completely automatically controlled.

A potentiometer cell has been described for indicating the chlorine content of solutions or to regulate the flow of chlorine from a tank to the absorbing solution (*335*). A calcium carbonate saturation balance indicator (*223*) gives a continuous indication of the potential difference between the sample and a separate portion of the sample which has flowed through calcium carbonate powder in a column long enough to ensure equilibrium (continuous reference sample). The degree of supersaturation of boiling sugar solutions has also been determined potentiometrically (*228*).

Currentimetry. The term currentimetry includes the measurement of the magnitude of a current which depends on the amount of a desired constituent present. Actually current is the measured quantity in other methods which are dependent on such properties as conductivity (resistivity), dielectric constant, potential difference, radioactivity, and those where photocell current is involved. However, only methods where the currents measured actually pass through and depend on the sample being investigated, or are carried by the desired constituent, are considered here.

Components in gaseous mixtures may be determined, according to a Russian patent (*481*), in an apparatus which detects them qualitatively by the value of their ionization potentials and determines them quantitatively by the increase of ionization current when the ionization potential of the given gas component is reached. A method for continuous estimation of oxygen in gases makes polarographic readings during the flow of gas through the cell (*551*) containing an electrolyte. Oxygen in lake waters is similarly determined. The potential is preset and the recorder then plots current against time (*334*).

A recent polarographic study with a microelectrode, past which an electrolyte is flowing, has been made (*366*). The stationary platinum microelectrode is sealed into the constricted part of a glass tube, past which the sample flows. Measurement reproducibility depends on constant rate of flow, constant rate and direction of change of applied voltage, constant temperature, careful pretreatment of electrode, and constancy of kind and concentration of supporting electrolyte. When conditions were properly controlled, the limiting currents obtained with the bypass electrode were shown to be a linear function of concentration of reacting substance, and log of rate of flow. It can be used to great advantage in the analysis of flowing systems—e.g., the blood stream.

Residual chlorine in water can be continuously recorded by the use of a rotating copper, platinum, or gold electrode (*336, 534*). One such method, which has been used by the Chicago Water Purification Division since 1942, is based on the depolarizing action of chlorine on a copper electrode (*31*). As molecules of hypochlorous acid reach the copper electrode, they depolarize it, causing a current to flow in the cell circuit proportional to the concentration of the acid. Frequent checks are necessary to ascertain the variations due to such factors as silver electrode deterioration, temperature, pH, combined chlorine, and dissolved salts. A fast continuous method such as this was required because the water in some Chicago stations reaches users in 1 to 2 minutes after it has been chlorinated, and ordinary routine tests were too late in their results.

An electric moisture meter (*211*) has been developed in which a constant alternating current voltage is applied to a sample contained in a small vessel which may be regarded as a fixed air condenser or in some instances as a conductivity cell. The current passing through the material is measured by a sensitive current (proportional to the dielectric constant), or the conductance current (proportional to the alternating current conductivity), or to some function of both these currents.

The measurement of the charge carried by equal-energy masses which have been separated by a magnetic field, as in mass spectroscopy, may be classed as a currentimetric method, as it is the current delivered to the collector plate that is measured

(*320*). If a material can be introduced into and removed from a mass spectrometer tube satisfactorily, the mass spectrometer has a very wide opportunity for doing both qualitative and quantitative analyses (*180, 293, 344, 378, 549*). A process control mass spectrometer (*175, 293*) is commercially available which will record up to eight mass concentrations nearly continuously. By programing the acceleration voltages over eight different values, a multipoint recorder can record the desired quantities.

Dielectimetry. The passage of an alternating current through materials, placed between the two plates of a condenser, is dependent on its dielectric constant, which in turn depends mainly on the water content.

Most devices which have been described use high-frequency currents (*15, 134, 318, 365, 460, 499, 550, 555*). The cell consists of a pair of conducting plates positioned so as to form the side walls of a housing adapted to contain the material to be tested, insulating material forming the bottom. Such instruments are used for moisture measurements on various granular, shredded, pulped, or powdered materials (*29, 489, 490*). The sample should be compressed to a point where further compression does not affect the electrical properties. One instrument commercially available in this country is designed for use with only two materials rather than for universal use (*390*). The basic instrument is adjusted by the makers to cover any moisture range at any level of moisture. Temperature influence, often considerable, is automatically compensated in the dial readings. A Russian report (*357*) states that it is applicable to the determination of water in salts and strong electrolytes, liquid air, sulfuric acid, and some organic liquids.

Radioactimetry. The emission or transmission of subatomic particles can form the basis of many kinds of quantitative measurements. Such methods have been recognized as capable of automatization, although widespread application has not yet been made. With increased availability of isotopes from United States atomic energy piles, there should be an increased amount of work in this field. A method of analysis of minerals in meteorites based on radiochemistry or "cosmic chemistry" is being studied (*5*).

Space here does not permit a description of the basic measuring instruments such as the various counters. The Geiger-Müller counter is the most commonly used type, but there are many variations (*240, 290, 375*).

Radioactive measurements of densities of liquids have been made in petroleum refineries (*207*). A highly penetrating beam from a source such as a mixture of radium and beryllium is directed inwardly through the wall of a pipe or other container and into the fluid under test, wherein the radiated particles are scattered in all directions. Those which are returned outwardly through the wall are detected and determined to give a measure of the density or specific gravity. A portable instrument (*282*) based on a similar phenomenon of back-scattered or reflected radiation makes the measurement by directing gamma rays at the material to be examined.

Bombardment by deuterons has been the principle of several methods, one for determining small amounts of carbon in iron and another of mercury vapor. The former (*13*) involves conversion of the carbon atoms to radioactive nitrogen 13, whose measured activity is a function of the carbon content of the sample. The method for mercury vapor (*184*) consists in the production of radioactive mercury 197 from gold and using it as a tracer in ordinary mercury. This technique offers promise of a more general application in problems of industrial hygiene.

The tracer technique (*485*) is applicable in industrial processes requiring repeated determination of a radioactive material, as it progresses through a sequence of operations in the course of which its concentration changes (*8, 45, 221*). One company (*516*) offers the service of developing a simplified rapid accurate automatic chemical analysis scheme for plant production work in which a radioisotope is introduced at the beginning of a process. Schreiber (*456*) has recently discussed the potentialities of radioisotopes in industry, with specific examples of how isotopes would be valuable in all stages of the viscose rayon process. He states that tens of thousands of potential industrial uses exist, since virtually every industry can profitably use the properties of one or more of the hundred-odd radioisotopes whose half-lives and availability permit their consideration for industry. Radiosulfur 35 is proposed as the proper tracer for enabling process control of the manufacture of rayon, including leakage, density, and liquid level measurement. Another suggestion for the determination of the uniformity and amount of sodium oleate on rayon yarn involves the use of sodium 24, which was mixed with the oil and sodium oleate before its application. Radioactive indicators should also prove useful in industrial hygiene problems in tracing dust and smoke fumes. Stanley's review evaluates the possibilities of tracer isotope uses in metallurgy for process control and analytical procedures. Although adequate automatic continuous indicating instruments have not yet been developed and proved which will give warning when leaks of many dangerous volatile materials occur, the use of tracer methods and strategically located radioactivity monitors should solve the problem in many cases. Recent work on the application of crystals to detect and count atomic radiation should prove fruitful, particularly because of their sensitivity and long life compared with the 3-month to 2-year life of conventional counters.

Safety measurements of radiation in work areas at Oak Ridge are accomplished by means of an ordinary traffic counter to give a record of the radiation at the counter with reference to time (*153*). The use of an automatic sample changer for routine radioassays on up to 25 samples has been mentioned (*516*). It is used with an autoscaler, which is a scaling circuit and precision timer designed so that, when a predetermined number of impulses has been counted, the timer is automatically stopped and the reading taken. A printing interval timer can be used in conjunction with the autoscaler to provide a printed record of radioassay results. It indicates up to 100 minutes in increments of 0.001 minute, and the sample number is printed in red beside the time.

Neutron spectroscopy has been studied in work connected with atomic energy research. A neutron absorption method has been developed for the analysis of boron and can be used to measure saturations in laboratory flow experiments. The latter arrangement allows for measuring oil, gas, and brine saturation in cores or sand packs. Neutrons passing through a hydrogen-rich substance are quickly slowed down, and the number of slow neutrons emerging from an object irradiated by fast neutrons gives a rough index of the quantity of hydrogen it contains and can be made very quantitative under controlled conditions (*65*). Neutrons may also prove useful for gas analysis. Unlike x-rays, neutrons are scattered more by the light elements than by the heavy ones.

Minute quantities of carbon 14 are measured by oxidation to carbon dioxide and used in turn as a gaseous filler for a counter to measure below 10^{-5} gram-mole of radioactive carbon dioxide with good accuracy (*232*).

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Direct-Recording Raman Spectrometer

J. J. HEIGL, B. F. DUDENBOSTEL, JR., J. F. BLACK, AND J. A. WILSON
Esso Laboratories, Standard Oil Development Company, Linden, N. J.

A photoelectric recording Raman spectrometer which provides rapid, accurate analyses of a wide variety of hydrocarbon samples has been in operation with a service factor of 95% for the past 2.5 years. The instrument employs a Toronto-type low pressure mercury excitation source, a Young-Thollon prism monochromator, and a refrigerated RCA-C-7073B photomultiplier detector which actuates a two-recorder system through a direct current amplifier. The background noise level is 1% of full-scale recorder deflection when operating at maximum sensitivity. At the sensitivity normally employed for the analysis of 4-ml. samples, repeated measurement of the 459 $\Delta\bar{\nu}$ cm.$^{-1}$ carbon tetrachloride peak indicates a reproducibility of better than 1% over a 50-minute period, the time required to obtain a complete spectrum. In making determinations of individual compounds in multicomponent synthetic blends, deviations of less than ±2 numerical % are observed.

FOR laboratories engaged in spectrographic work, a wide variety of commercial instruments is now available for direct-recording or point-reading measurement of ultraviolet, visible, and infrared absorption spectra. In this general development, however, the Raman effect has not received attention equal to that expended on infrared or ultraviolet methods of analysis. The principal reason for this lack of interest was the necessity of employing a cumbersome and slow photographic technique for recording and measuring the low intensity radiation involved in the Raman effect.

However, Raman spectrographic techniques have recently become practical for many analytical applications because of the availability of photoelectric methods to measure low-level illumination. Studies at this laboratory and elsewhere show that many multicomponent mixtures can now be analyzed with greater speed and accuracy by Raman spectrometry than by any other known method.

The application of photoelectric detection to Raman spectrometry by Fenske, Rank, and Wiegand (*2*, *4*, *5*) has demonstrated the practicability of the construction of accurate, direct-recording instruments which render Raman spectra as convenient as other optical methods for rapid analytical work. A photoelectric recording Raman spectrometer constructed at the Esso Laboratories of the Standard Oil Development Company has been in use for the analysis of hydrocarbon mixtures for the past 2.5 years. In a 50-minute operating period, this instrument provides a direct plot of a Raman spectrum for wave-length shifts from 50 to 1700 cm.$^{-1}$ Highly reproducible measurements can be performed with a sample as small as 4 ml. The design and operation of this spectrograph are discussed in this paper.

DESIGN AND CONSTRUCTION

The Raman spectrometer described in this paper was designed to furnish an instrument which could be used for rapid and accurate routine analyses of complex hydrocarbon mixtures. It has been shown by Fenske *et al.* that a resolution of 10 to 15 cm.$^{-1}$ is satisfactory for the analysis of hydrocarbon mixtures by Raman spectrometry; this indicates that the design of the monochromator is not highly critical. Emphasis was placed on the mechanical and electronic design to achieve the required high degree of stability and reproducibility.

A description of the component parts of the instrument, shown photographically in Figure 1, is presented in the following sections of this paper.

Light Source and Sample Tube Assembly. For the excitation of Raman spectra of small samples, the excitation source must furnish maximum illumination of uniform intensity. Although not important in photographic Raman spectroscopy, uniform intensity is of great importance in the photoelectric method. Other requirements of the source include a minimum of continuous background radiation, low intensity of all but the 4358 A. exciting line, and a long operating life.

The characteristics of a high pressure and a low pressure mercury vapor arc have been studied extensively at these laboratories.

The high pressure arc included in this program was the Model H-1 arc, manufactured by the General Electric Company. The low pressure arc was supplied by the Physics Department at the University of Toronto. A comparison of these two sources has shown that the low pressure Toronto source is superior in all respects to the high pressure source. The Toronto type source is now being employed exclusively for Raman excitation at the Esso Laboratories.

A photograph of the Toronto source, presented in Figure 2, shows the arrangement of the source and sample tube assembly. The source consists of a four-turn, 8.9-cm. (3.5-inch) diameter helix. One-inch diameter Pyrex tubing is used in the construction of the helix. The two mercury-filled electrodes, fitted with tungsten connections, are water-cooled. Also shown in the photograph are the infrared heating lamps and a spark coil, both of which are used in the initial firing of the arc. On starting, the arc requires from 200 to 250 volts (direct current) and approximately 85 volts thereafter. A current of 15 amperes is employed during operation. The wiring diagram of the source unit is shown in Figure 3.

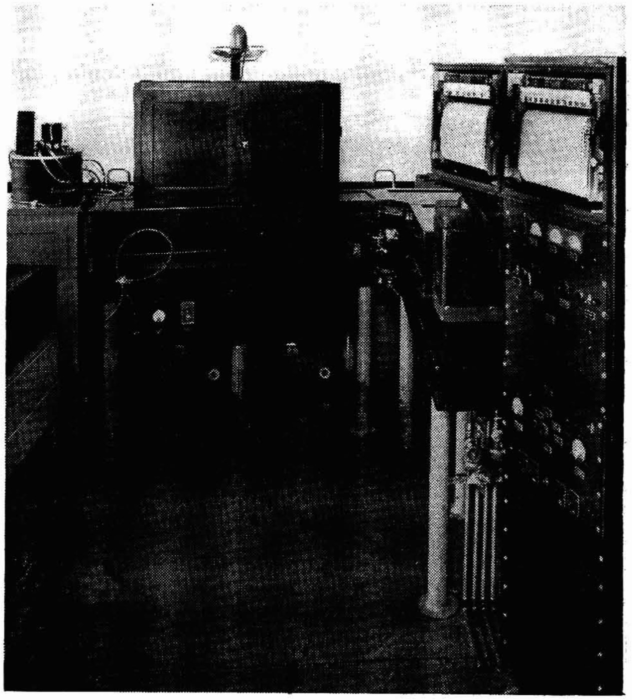

Figure 1. Photoelectric Pen Recording Raman Spectrometer

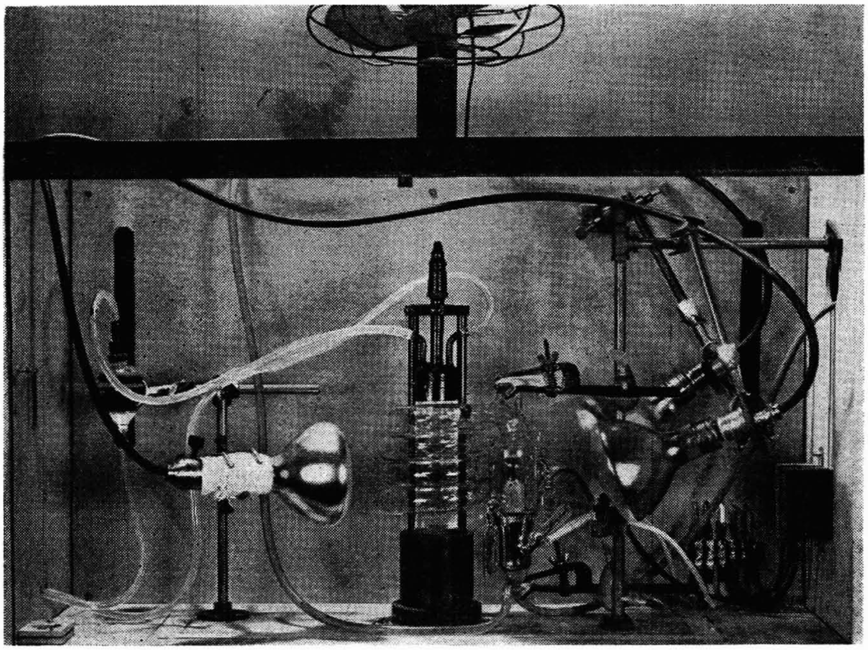

Figure 2. Raman Excitation Source Assembly

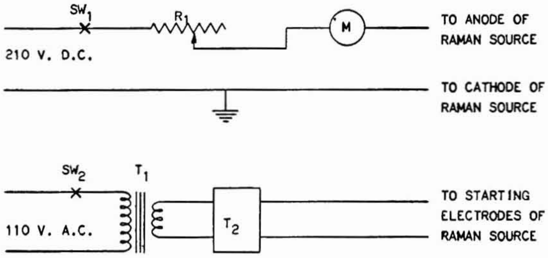

Figure 3. Circuit Diagram of Source Power Supply

R_1.	Rheostat, wire-wound, water-cooled, 20 ohms
M.	Ammeter, 0 to 30 amperes
SW_1.	S.P.S.T. switch
SW_2.	Switch, momentary contact
T_1.	Transformer 115-volt primary, 6.3-volt secondary
T_2.	Spark coil

The sample tube, designed in accordance with the specifications given in Figure 4, is constructed from Pyrex. The inner cylinder, which holds the sample, has a volume of 4 ml. This size of sample tube was chosen because the amount of liquid sample available for many of the analyses in hydrocarbon research is limited to about 5 ml. In other applications, where a larger amount of sample is available, a larger sample tube is advantageous, permitting measurement at a greater signal-to-noise ratio. A saturated sodium nitrite solution is circulated through the jacket surrounding the sample tube. The temperature of the filter solution is controlled at 85° F. The purpose of the filter solution is to reduce unwanted radiation emanating from the mercury 4047 and 4078 A. lines.

During operation, cooling of the source and sample tube assembly is effected by a standard electric fan, the air stream from which is directed downward onto the top of the source.

Monochromator. The monochromator is designed to scan the spectrum between approximately 5145 and 4369 A., corresponding to wave-number shifts of 3500 to 50 cm.$^{-1}$ from the 4358 A. exciting line.

The optical system has an aperture of $f/8.8$ and a dispersion of 23 A. per mm. (at 4861 A.). In the dispersing system, shown in Figure 5, three prisms are utilized in a Young-Thollon type mounting. The prisms are constructed from DF-2 glass which has high dispersion in the violet region of the visible spectrum where scanning is carried out. The prism assembly includes two 30° and one 60° prisms with a total base width of 150 mm. and a height of 60 mm. The 60° prism is mounted on a stationary pedestal; the 30° prisms are mounted on kinematically designed tables which are rotated by the motor-driven wedge. The wedge is also mounted in accordance with accepted kinematic principles.

Radiation from the sample tube is focused onto the entrant slit by a condensing lens. A 90° prism is used in this system to deflect the beam from the sample cell onto the entrant slit. After passing into the monochromator, a schematic diagram of which was given in a previous publication (*3*), the light falls on the collimator lens and as a parallel beam fills the aperture of the dispersing assembly. The dispersed radiation is focused by the camera lens onto the curved exit slits. The collimating and camera lenses are cemented achromats which, in addition to all other optical components, were coated with magnesium fluoride to reduce reflectivity losses.

Of particular importance is the amount of slit curvature, so that a perfect match between the Raman line image and the slit is attained. To obtain this condition, the exit slits were curved by a hand-honing operation. The 4358 A. mercury line was used as a reference and the curvature of the exit slits matched to the curvature of this line at the focus of the camera lens.

As the Raman lines are moved successively across the exit slit by motion of the motor-driven wedge, they are focused on the photosensitive element of the photomultiplier detector by two lenses.

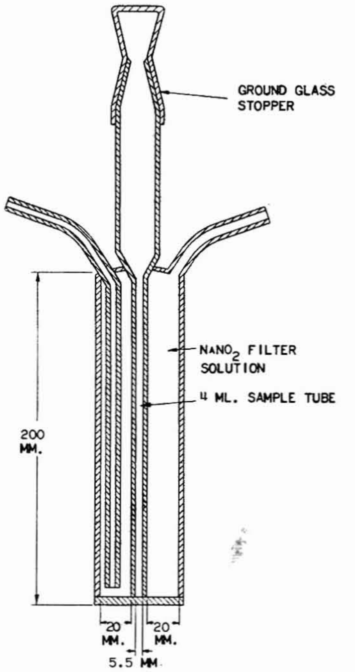

Figure 4. Raman Sample Tube

Detector Assembly. Detection of Raman lines is performed by a refrigerated photomultiplier tube. The cooling medium employed in the present instrument is dry ice. Cooling of photomultipliers has been reported (*1*) as a method for improving signal-to-noise ratios at low light levels. A schematic diagram of the photomultiplier housing was presented in a previous publication (*3*).

The particular photomultiplier found most satisfactory is an RCA-C-7073B, selected from a series of experimental tubes of this type. These tests were carried out in cooperation with research personnel at the Radio Corporation of America, manufacturer of the photomultiplier tube.

The accelerating voltages applied to the successive stages of the photomultiplier are supplied by a series type regulated power supply containing two voltage amplifiers, the schematic diagram of which is given in Figure 6. This supply has been found more constant than a battery supply which was previously employed and has required less maintenance. The supply voltage to the photomultiplier is properly proportioned to each stage by re-

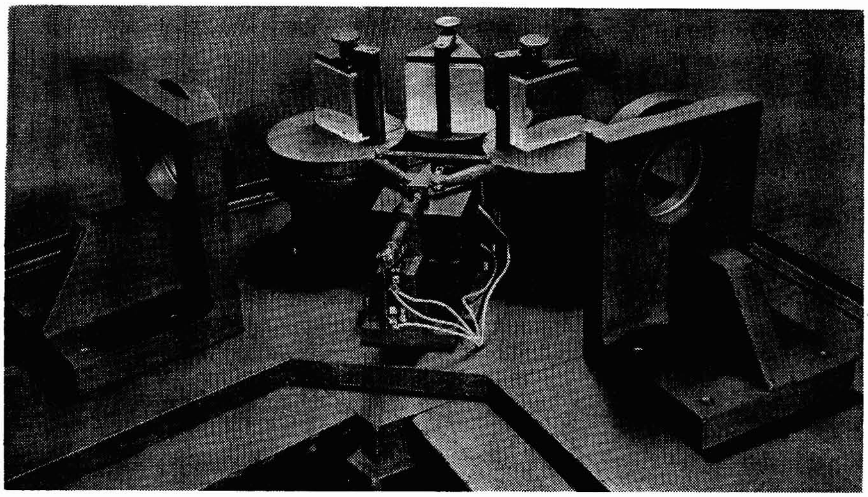

Figure 5. Dispersing Assembly

sistors in a voltage dividing network, shown schematically in Figure 7, mounted adjacent to the photomultiplier in the refrigerated housing (Figure 8). For optimum signal-to-noise operation, 40 volts per stage are found preferable. A higher voltage, between 65 and 90 volts, is impressed between plate 9 and the dynode, electrode 10. The output from the photomultiplier is connected through a low-loss cable to the amplifier described in the following section of this paper.

Amplification System. The schematic diagram of the amplifier which was built at these laboratories for amplification of the photomultiplier output is given in Figure 9.

The design of the amplifier closely follows that of the Leeds & Northrup thermionic amplifier which is a current amplifier employing an RH 507 electrometer tube. Switch SW_1 (Figure 9) in the grid circuit of the electrometer tube was constructed to meet the low electrical leakage requirements encountered in high resistance circuits. Leakage across the switch, resistors, and electrometer tube envelope is minimized by enclosing these components in an air-tight box with a desiccant. An input resistance of 10,000 megohms is most generally used and makes possible the measurement of amplifier input currents as low as 3×10^{-14} ampere. Low-loss mica condensers (C_1, C_2, and C_3 in Figure 9) in conjunction with the grid resistors cause the amplification system to operate with a time constant of a few seconds, thereby improving the signal-to-noise relationship of the recorded voltage.

Power is supplied to the amplifier by wet-cell batteries (Willard), which are recharged at monthly intervals.

The drift of the amplifier is low, amounting to less than 1% of full output signal per hour.

Recording System. For both qualitative and quantitative analytical applications, it is desirable to record a wide range of Raman peak intensities with maximum accuracy. This requirement has been met by operating two recorders at sensitivity ratios of approximately 1 to 3.

Although any fast electronically operated recorder is probably satisfactory for the application, Brown Electronik recorders were employed. The full-scale sensitivity selected for the more sensitive recorder is approximately 60 mv. Both recorders have been modified to operate through a 10,000 ohm–5 microfarad input circuit rather than the 200 ohm–500 microfarad circuit with which they were originally equipped. In the range-selecting system, the recorder operating at the higher sensitivity is disconnected from the circuit when the less sensitive recorder is operat-

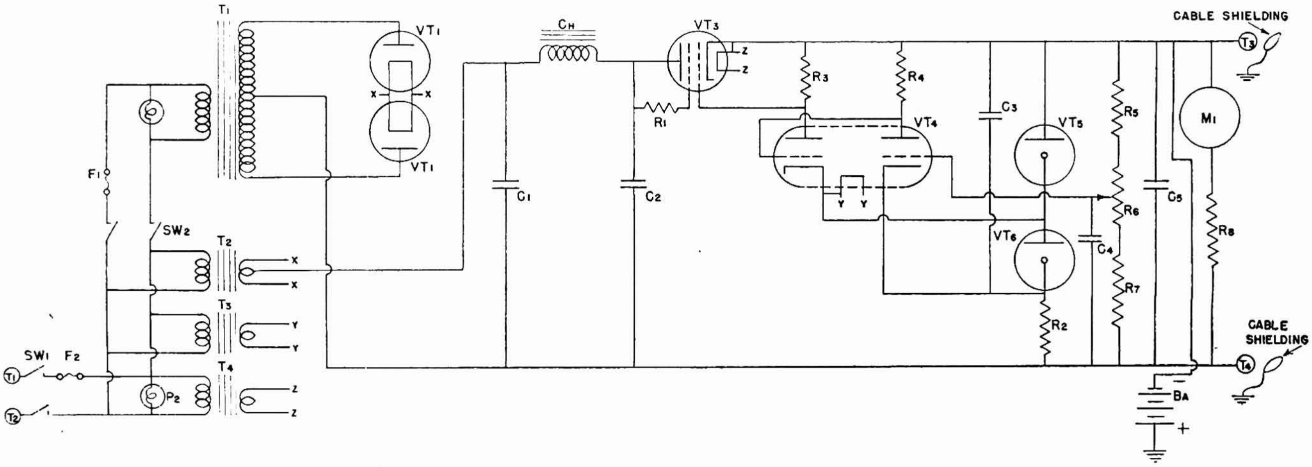

Figure 6. Regulated Power Supply for Photomultiplier

Components

T_1.	110-volt-600-0-600-volt transformer
T_2.	110-volt a.c.–2.5 volt, 10-ampere transformer
T_3.	110-volt a.c.–6.3 volt, 3-ampere transformer
T_4.	110-volt a.c.–6.3 volt, 3-ampere transformer
R_1.	100-ohm 2-watt resistor
R_2.	20,000-ohm 10-watt resistor
R_3.	500,000-ohm 1-watt resistor
R_4.	500,000-ohm 1-watt resistor
R_5.	50,000-ohm 10-watt resistor
R_6.	10,000-ohm 4-watt potentiometer
R_7.	20.000-ohm 10-watt resistor
R_8.	500,000-ohm precision resistor
C_1.	4-mfd. 1000-volt oil condenser
C_2.	4-mfd. 1000-volt oil condenser
C_3.	0.25-mfd. 600-volt paper condenser
C_4.	2.0-mfd. 1000-volt oil condenser

Components

C_5.	2.0-mfd. 1000-volt oil condenser
C_h.	8–10-henry, 150-ma. choke
P_1.	110-volt red panel pilot
P_2.	110-volt green panel pilot
F_1.	Panel mount fuse 2 amp.
F_2.	Panel mount fuse 3 amp.
VT_1.	1616 tube
VT_2.	1616 tube
VT_3.	807 tube
VT_4.	6SL7 tube
VT_5.	VR150 tube
VT_6.	VR150 tube
SW_1.	S.P.D.T. toggle switch
SW_2.	S.P.D.T. toggle switch
M_1.	0–1 ma., 3-inch meter
Ba.	67.5-volt battery (for last stage)

Terminal connections

T_1.	110-volt a.c. 60 cp. power
T_2.	110-volt a.c. 60 cp. power
T_3.	Positive output high voltage connector
T_4.	Negative output high voltage connector

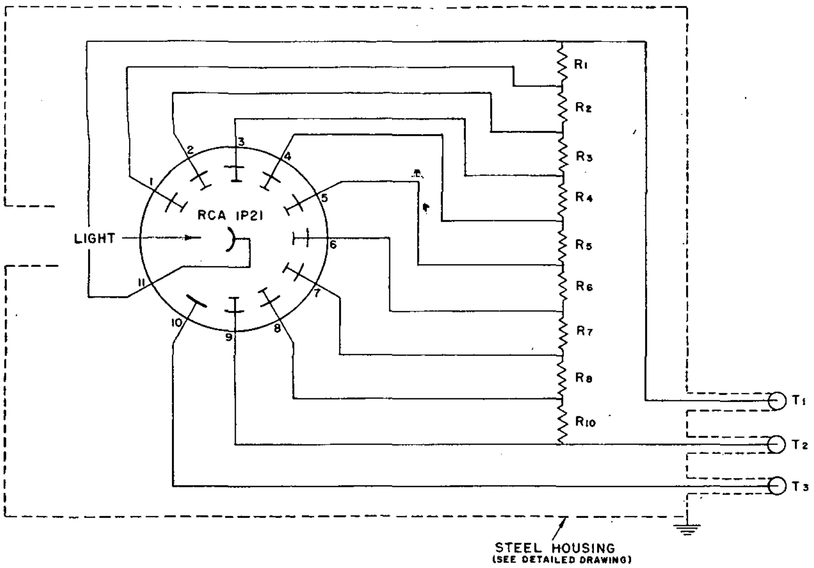

Figure 7. Photomultiplier Circuit

Components
R_1 to R_9. 0.5-meg. precision resistors
RCA IP21. See Figure 8 for mounting details
Terminal connections
T_1. Negative connection of 440-volt regulated d.c.
T_2. Positive connection of 440-volt regulated d.c.
T_3. High impedance output connection to RH 507 d.c. amplifier grid input
Cable details
T_1, T_2. To supply high tension ignition cable in copper sheathing
T_3. Polyethylene coax cable, 1-kw. rating, amphenol low-loss coax connector

Table I. Reproducibility Test

(Sample, carbon tetrachloride. Sample size, 4 ml.)

Test No.	Peak Height, Mm. Scan 1	Peak Height, Mm. Scan 2	% Deviation
1	112.0	111.5	0.4
2	109.5	109.0	0.5
3	109.0	109.0	0.0
4	108.5	110.0	1.4
5	111.0	111.2	0.2
6	111.0	111.8	0.7
7	111.0	110.7	0.3
8	110.5	110.9	0.4
9	107.5	108.2	0.7
10	108.5	109.3	0.7
		Mean	0.5

ing above 33% of full scale. Switching is carried out by means of a Microswitch, which is actuated by a cam mounted on the pen cable-drive drum of the less sensitive recorder.

Fiducial marks are produced by injecting a low-voltage, short-time pulse into the recorder standardizing circuit. The pulse is initiated by a Microswitch operated by a notched disk mounted on the wave-length drive mechanism. Further details of the recorder and wave-length marking circuit are presented schematically in Figure 10.

OPERATING CHARACTERISTICS

The type of spectrum recorded by the spectrometer is shown in Figure 11. This trace is a direct photographic reproduction of a spectrum obtained utilizing a 4-ml. sample. Approximately 50 minutes are required to scan the 50 to 1650 cm.$^{-1}$ range covered by this spectrum. Freedom from variation in background, over the region scanned, makes the spectra particularly useful not only for qualitative but also for quantitative analyses. In quantitative analyses, satisfactory accuracy can be obtained for only a limited number of hydrocarbon mixture types, if background variations exist. Also of importance is the minimizing of all Raman peaks due to excitation by mercury lines other than the 4358 A. radiation. That this condition is attained is demonstrated by the spectra being recorded by the present instrument.

Because the principal application of the instrument is in service in which high reproducibility is a primary requisite, no attempt is made to operate at conditions of maximum resolution. The spectral slit widths being employed are 11 cm.$^{-1}$ (at 4861 A.); which have been found sufficient for all present analytical applications at these laboratories. Data show that increased resolution can be attained at the smaller slit sizes; these reduced slits will be employed if analytical problems arise which require greater separation of spectral lines.

In quantitative application of Raman spectra, constancy of resolution over an extended period is desired. Should the resolution change, a considerable amount of recalibration would be required. Several weeks' work is ordinarily entailed in rerunning the pure compounds in such a recalibration procedure. Variations in instrument adjustment which may result in a change in resolution include variation in source lamp characteristics, shifting of the optical alignment, and change in the photoemissive sensitivity of photomultipliers.

The reproducibility of the instrument, measured by its ability to scan a Raman line repeatedly, is better than 1% over a 1-hour period. Data in Table I show the reproducibility obtained in scanning the carbon tetrachloride peak at $459\,\Delta\bar{\nu}$ cm.$^{-1}$ over a 2-week period. An interval of 1 hour elapsed between scans 1 and 2.

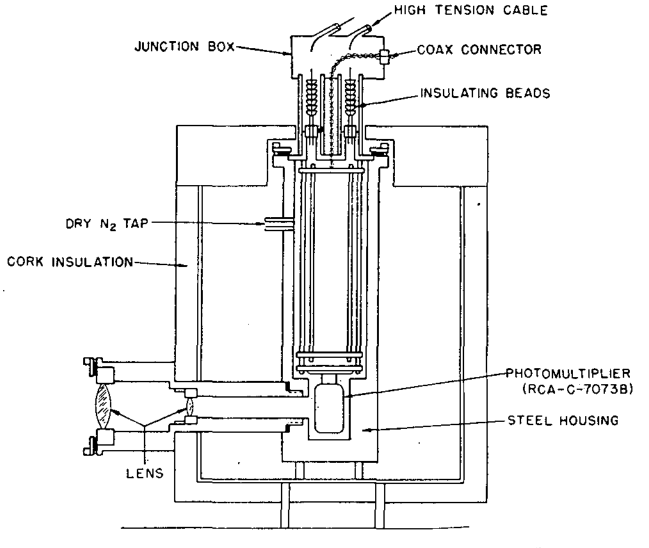

Figure 8. Schematic Drawing of Photomultiplier Housing

No variation in scattering coefficient has been observed over an extended period of operation. Data given in Table II show that the change in scattering coefficient over a period of approximately 1 month amounted to less than 3%, with the exception of one value.

Linearity. Quantitative analyses require that a linear proportionality be maintained between the recorded peak height and the number of irradiated molecules producing that peak. This linear relationship must be obtained at all settings of amplifier gain. The first step employed to check linearity consists in scanning a

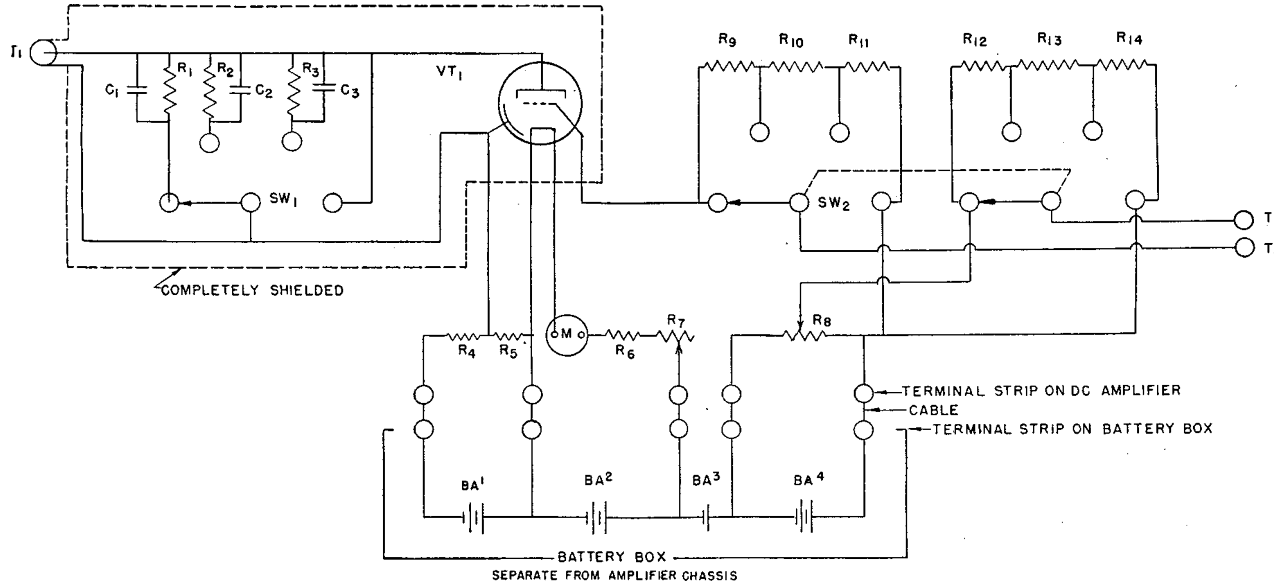

Figure 9. Direct Current Amplifier

Components

R_1.	10,000 megohms
R_2.	2000 megohms
R_3.	100 megohms
R_4.	2000 ohms precision resistor
R_5.	7500 ohms precision resistor
R_6.	25 ohms precision resistor
R_7.	15 ohms w.w. potentiometer
R_8.	330 ohms w.w. potentiometer
R_9.	5000 ohms precision resistor
R_{10}.	4000 ohms precision resistor
R_{11}.	1250 ohms precision resistor
R_{12}.	5000 ohms precision resistor
R_{13}.	4000 ohms precision resistor
R_{14}.	1250 ohms precision resistor

Terminal connections

T_1.	Amphenol low-loss coax connector to connect to coax cable from detector
$T_2 + T_3$.	Recorder connections, 2 wire shielded cable connector

Components

C_1.	0.001-mfd. mica condenser
C_2.	0.005-mfd. mica condenser
C_3.	0.01-mfd. mica condenser
VT_1.	Westinghouse RH-507
M.	0–100 ma. meter
SW_1.	Special low-loss switch
SW_2.	6-position 2-pole Yaxley rotary switch
BA^1.	4-volt Willard low drain cells (2)
BA^2.	4-volt Willard (1-amp. capacity) low drain cells (2)
BA^3.	2-volt Willard low drain cell
BA^4.	4-volt Willard low drain cell

Raman peak at various sample volumes. The constancy of the peak height-to-sample volume ratio is one indication of the linearity of the instrument. Data in Table III show this ratio for both the high and low sensitivity recorders in three scanning operations in which carbon tetrachloride was used. These values indicate that the linearity is satisfactory for quantitative applications.

Table II. Reproducibility of Scattering Coefficients

(Sample, toluene)

Raman Displacement, Cm.$^{-1}$, of Major Lines	Scattering Coefficients[a] 12/20/48	Scattering Coefficients[a] 2/1/49	% Deviation
1604	0.332	0.330	0.6
1208	0.153	0.157	2.6
1030	0.312	0.309	1.0
1004	1.10	1.06	3.6
786	0.573	0.580	1.2
730	0.138	0.140	1.4
521	0.227	0.230	1.3
216	0.317	0.312	1.6

[a] Relative to 459 $\Delta\bar{\nu}$ cm.$^{-1}$ CCl_4 line.

Table III. Linearity of Raman Spectrograph

(459 $\Delta\bar{\nu}$ cm.$^{-1}$ carbon tetrachloride, Raman line)

Sample Volume, Ml.	High-Sensitivity Recorder		Low-Sensitivity Recorder	
	Signal, mm.	Signal-sample ratio	Signal, mm.	Signal-sample ratio
3.8	249	65.6	80.0	21.0
8.8	577	65.6	186.0	21.1
18.0	1184	65.8	382.0	21.2

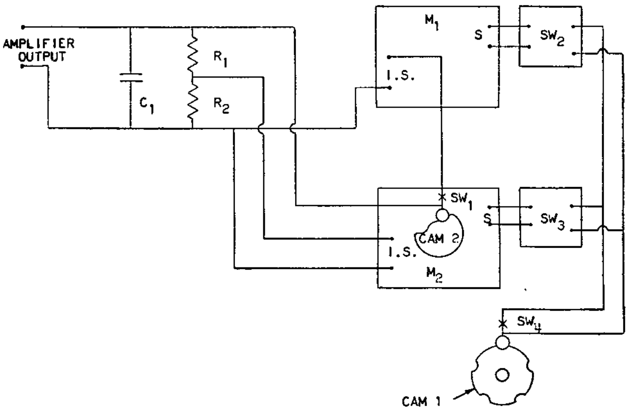

Figure 10. Recorder and Wave-Length Marking Circuit

C_1.	Condenser, 5-mfd. 600-volt, oil filled
R_1.	Resistor, 6800 ohms, wire wound
R_2.	Resistor, 3300 ohms, wire wound
M_1, M_2.	Brown Electronik high speed recorders
I.S.	Input circuit (500-mfd. condenser normally present is disconnected)
S.	Standardizing circuit
CAM 2.	Cam mounted on lower sensitivity recorder to cut off M_1 by means of SW_1
SW_1.	Microswitch
SW_2-SW_3.	Vacuum tube relays
SW_4.	Microswitch actuated by cam 1
CAM 1.	Cam mounted on wave-length drive shaft

The second step in checking linearity consists in determining the magnitude of the difference between the peak height obtained by scanning and by stopping the wave-length drive when the top of the peak is reached. The percentage of full peak height recorded should be at least 99% and has been achieved with the instrument described in the present paper. At a recording speed of approximately 33 cm.$^{-1}$ per minute, a filter with a 5-second time constant has been found optimum.

Analytical Accuracy and Reproducibility. The most conclusive test of the performance of any analytical instrument is its

Table IV. Analysis of Synthetic Blends by Raman Spectrometry

Components in Blend	Composition, Vol. % Known	Composition, Vol. % Reported	Numerical Deviation, %
Synthetic A			
1,3,5-Trimethylbenzene	6.1	5.4	−0.7
o-Ethyltoluene	6.1	5.0	−1.1
1,2,4-Trimethylbenzene	61.0	62.6	+1.6
tert-Butylbenzene	3.0	4.1	+1.1
1,2,3-Trimethylbenzene	17.2	17.9	+0.7
Hydrindene	6.6	5.0	−1.6
Synthetic B			
n-Heptane	47.9	47.1	−0.8
3-Methylhexane	24.5	24.2	−0.3
trans-1,2-Dimethylcyclopentane	10.4	10.1	−0.3
cis-1,3-Dimethylcyclopentane	10.4	11.1	+0.7
trans-1,3-Dimethylcyclopentane	4.9	4.9	±0.0
cis-1,2-Dimethylcyclopentane	1.9	2.6	+0.7

accuracy and reproducibility in the analysis of synthetic blends of the type that will be encountered in the final application of the equipment. A large number of synthetic samples have been analyzed with the present instrument. The results of these tests show that, in general, the reproducibility and accuracy of the equipment are satisfactory. Data in Table IV show the results obtained for two synthetic samples, containing six hydrocarbons. Synthetic A, consisting of aromatic hydrocarbons, was analyzed at the time when the instrument was equipped with a high pressure mercury arc. Synthetic B contains paraffinic and naphthenic hydrocarbons, and was analyzed later, after the low pressure Toronto type source was installed. This mixture could not be analyzed satisfactorily with the high pressure source, which did not provide enough power sufficiently to excite the Raman lines of low intensity. Previously reported data (*3*) show that the determination of total aromatic and total olefin content of hydrocarbon mixtures can be performed with satisfactory accuracy. The reproducibility data show that the accuracy of measurement is retained, making frequent recalibration unnecessary.

Maintenance. The Raman spectrometer described in this paper has been in continuous operation for the past 2.5 years, during which time the service factor has been about 95%. The life of the source is unknown; it has been in operation 850 hours to date. Normal maintenance operation includes dry ice charging of the photomultiplier housing once per 8-hour day, charging of the amplifier batteries every 4 weeks (carried out over a weekend), and occasional cleaning, oiling, and checking of the recorders.

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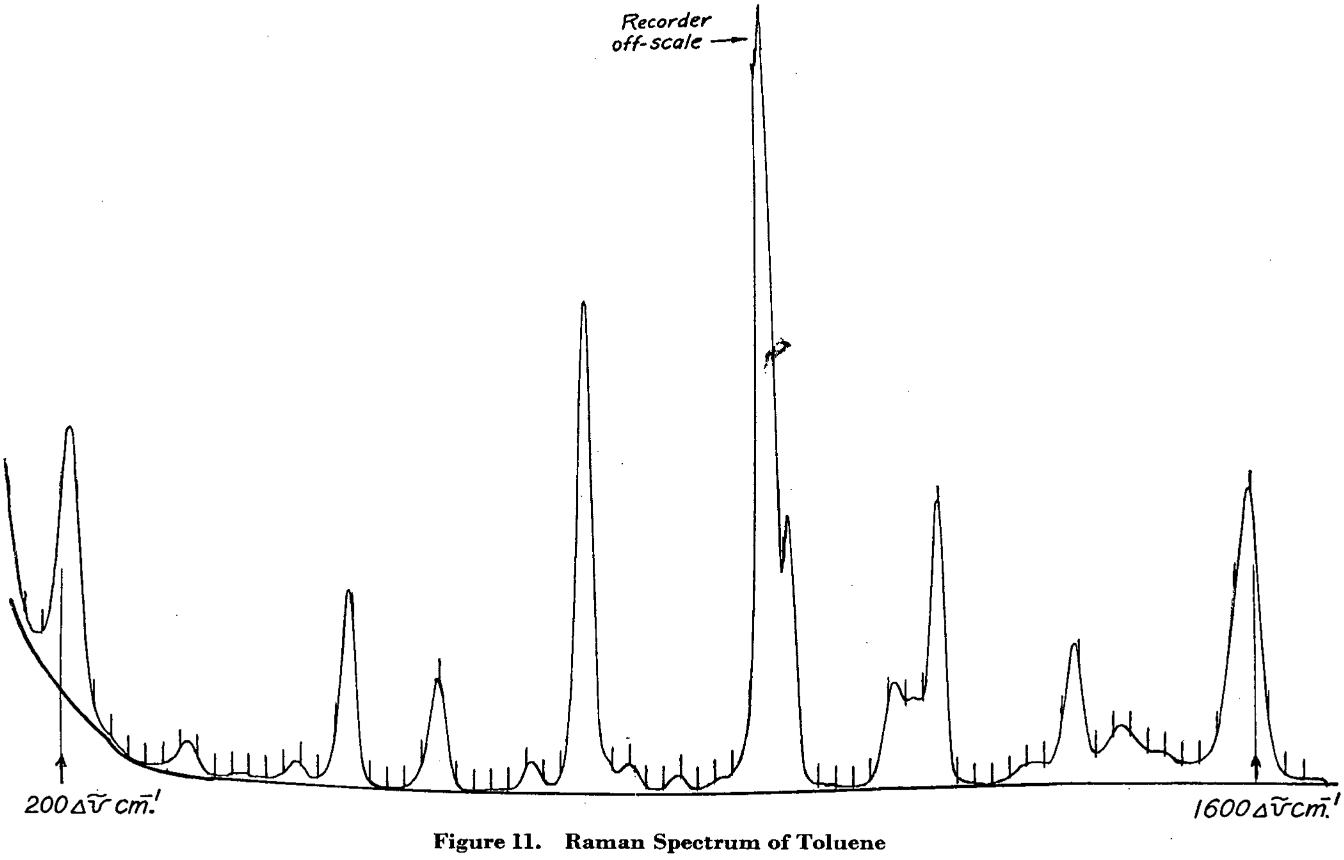

Figure 11. Raman Spectrum of Toluene

Differential Analysis with a Beckman Spectrophotometer

ROBERT BASTIAN, RICHARD WEBERLING, AND FRANK PALILLA
Sylvania Electric Products, Inc., Kew Gardens, N. Y.

The differential method of colorimetric analysis as applied to the Beckman Model DU spectrophotometer is discussed. The maximum increases in accuracy of this method over the normal one are computed for the instrument. Taking special precautions, experimental accuracies and precisions in the range of about ±0.5 to ±1 part per thousand are obtained on potassium dichromate and potassium permanganate solutions.

THE principles of differential colorimetry have been recently discussed (*1, 2, 6*). Utilizing the differential method on a Beckman spectrophotometer, Bastian (*2*) obtained concentration accuracies and precisions of 1 to 3 parts per thousand in the determination of copper. As indicated in his paper, however, these results cannot be expected to represent the maximum precision obtainable on this instrument. To the important sources of error considered there may be added those due to the various analytical manipulations as well as fluctuations in final acidity which might have affected the color intensities.

This paper discusses the differential method more thoroughly, computes the maximum accuracy (compared to the normal procedure) which can be obtained at various wave lengths on the Model DU Beckman spectrophotometer utilizing the method, and gives a better notion of the experimental accuracy and precision that can be obtained under carefully controlled conditions. In order to keep errors due to the formation of the colored product at a minimum, this work was conducted on aqueous solutions of potassium dichromate and potassium permanganate. To minimize volumetric errors aliquots were taken by weight wherever advisable. To eliminate errors due to calibration of volumetric equipment the same volumetric flask was used for diluting all standards and unknowns.

As previously described (*2*), in the differential method one sets the optical density scale zero (or the transmittancy for 100%) with a solution of a highly colored or light-absorbing substance in a place of a reagent blank. Higher concentrations of the given substance are then read against this zero point in the usual way. The optical density scale readings thus obtained represent the differences in absorbancy between the zero point samples and the samples in question. (In the new nomenclature, *9*, "absorbancy of the sample," A_s, replaces the term "optical density." $A_s = \log_{10} \frac{1}{T_s}$, where $T_s = \frac{T_{\text{solution}}}{T_{\text{solvent}}}$. The authors will use these newer terms but, because their Beckman instrument is marked in accordance with the older nomenclature, they will continue to use the term "optical density scale.") In order to obtain the increased light needed to set the scale zero by this procedure, the spectrophotometer slit is used at wider apertures than normal.

The effect of the procedure is shown in Figure 1. Here varying concentration ranges of permanganate, expressed in grams of a standard solution per indicated volume, were plotted against optical density scale readings.

For curve 1, distilled water was used to set the scale zero, so that the readings represent the true absorbancies of the samples; for curves 2, 3, and 4 successively increasing concentrations of permanganate were used to set the scale zero, so that the readings represent differences in absorbancy between the given samples and the zero point samples. All readings were taken with the sensitivity knob set 2 turns from the clockwise end, at a wave length of 526 millimicrons, using 1-cm. cells.

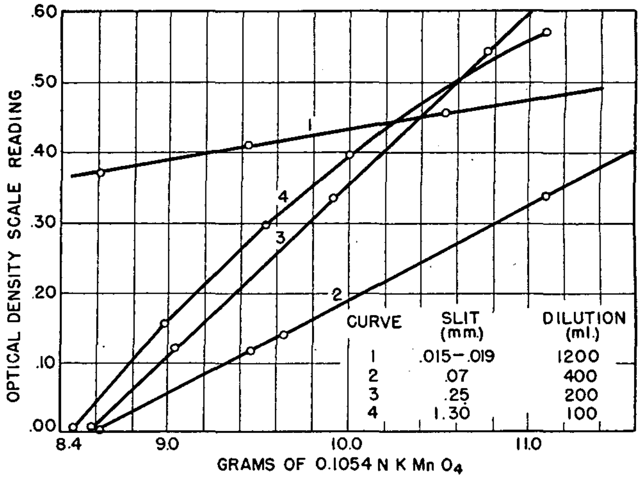

Figure 1. Effect of Spectrophotometric Procedure

As the horizontal axis indicates, the samples were prepared by weighing out appropriate amounts of a standard permanganate solution (1 N = 0.2 M) on an analytical balance. All the samples were actually diluted in a single 200-ml. volumetric flask which also served as container for the weighing. The concentration ranges are expressed in terms of a uniform weight scale per the indicated volumes to permit an easier visual comparison from the standpoint of relative accuracies. For curve 3 the weights shown are the weights actually taken; for curves 1, 2, and 4 one sixth, one half, and twice the indicated weights were diluted to 200 ml. This procedure of weighing aliquots permitted preparation of all necessary solutions with convenience and high accuracy.

Curves 1, 2, and 3 are straight lines with slopes greatly increasing as higher concentrations of color are used to set the zero point on the scale.

Qualitatively it can be seen that increasing slopes represent increasing accuracies in determining concentrations, but the subject can be treated more accurately as follows:

The error in determining concentrations, which is normally expressed as $\Delta C/C$, can be expressed in terms of the curves shown as $\Delta G/G$, where G is the number of grams of the permanganate solution corresponding to a given scale reading and ΔG is the minimum difference in G that we can detect at that point. If we let S = the slope of the line in question, (dA_s/dG), and ΔA_s the minimum difference in the absorbency of the sample that we can detect, it can be seen that:

$$\Delta G = \Delta A_s/S$$

or

$$\Delta G/G = \Delta C/C = \Delta A_s/S\,G \tag{1}$$

Comparing the concentration error of curve 1 to curve 2

$$\frac{\text{Error of 1}}{\text{Error of 2}} = \frac{\text{accuracy of 2}}{\text{accuracy of 1}} = \frac{\Delta A_{s1}\,S_2\,G_2}{\Delta A_{s2}\,S_1\,G_1} \tag{2}$$

If we compare the relative accuracies at a given point on the scale and assume that the reproducibility in reading the scale at this point is the same regardless of the concentration of color taken to set the scale zero ($\Delta A_{s1} = \Delta A_{s2}$), then:

$$\frac{\text{Accuracy of 2}}{\text{Accuracy of 1}} = \frac{S_2\,G_2}{S_1\,G_1} \tag{3}$$

The relative accuracies depend on the scale reading taken for comparison. Arbitrarily choosing 0.40, we find that at this point curve 2 yields 3.8 times the accuracy of 1 and curve 3 yields 5.5 times the accuracy of 1.

Curve 4 is not a straight line, its slope falling off considerably at the upper end of the range shown. In order to evaluate the accuracy here the same procedure could be followed, except that one would have to draw a tangent to get the slope at the desired point. One can see that at 0.40 curve 4 represents a lower accuracy than 3, for the slope is less and G is less. The accuracy of 4 with respect to 3 becomes much less as we pass to readings above 0.40. From the manner in which the slope of 4 is falling off it is evident that employing a still higher concentration range would result in a decreased, rather than increased, accuracy. It is evident that the differential method as described here has limitations imposed by:

A. The maximum concentration of colored (or light-absorbing) substance which the available light will penetrate sufficiently to permit setting the scale zero. Without disturbing the light source on the instrument, this is limited by the maximum slit aperture (2.0 mm.). Because different slit apertures are required for normal operation at different wave lengths, the increased light that a maximum aperture slit yields also varies with the wave length.

B. Deviations of working curves from Beer's law in the direction shown by curve 4. These deviations may arise for several reasons, among which are (*a*) too high a concentration of color, (*b*) too great a band width of light, and (*c*) stray light.

Using the instrument as described, *a* and *b* are bound together because a higher concentration of color requires a wider slit aperture and this in turn increases the band width of light which emerges.

As pointed out by Ayres (*1*), there are some systems which deviate from Beer's law in such a way as to give accuracy greater than that represented by a straight-line function, even by normal colorimetry. This occurs when the plot of absorbency *vs.* concentration curves upward. Naturally, differential colorimetry could be used for such systems, but these systems are comparatively rare.

The application of differential colorimetry to systems that deviate from Beer's law in the usual manner depends upon the extent of the deviations, but in general would be limited.

COMPUTATIONS OF ACCURACY

It is desirable to be able to compute relative accuracies in a different, if less exact, manner than that already described. Make the following assumptions concerning the given system:

1. The plot of optical density scale reading against concentration is linear over each concentration range selected for comparison.
2. A given increment in concentration always gives the same increment in absorbency over the entire range of concentrations. This does not follow from assumption 1 because each higher concentration range is measured at a wider slit aperture which yields a wider band width of light. If we set our instrument at the wave length of maximum absorption for the given substance, and the substance has a sharp absorption peak, increasing the band width will give a somewhat lower absorption reading. Thus, although each concentration range may give a straight-line plot, the slope of each higher range will be somewhat less than expected. If the color has a broad absorption peak, the effect is much less. The assumption made then is that the region of maximum absorption is broad enough to make assumption 2 valid over the band width range used. Data presented below for potassium dichromate and potassium permanganate tend to conform this reasoning.
3. The optical density scale is read at the same point in comparing relative accuracies.
4. The reproducibility in reading the optical density scale at any given point is independent of the concentration of the solution used to set the scale zero (independent of slit aperture). Data are given below to support assumption 4.

From the above assumptions it follows that:

$$\frac{\text{Accuracy of differential method}}{\text{Accuracy of normal method}} = \frac{A_s \text{ for differential solution}}{A_s \text{ for normal solution}} \tag{4}$$

Although it is not possible to obtain A_s for the differential solution with sufficient accuracy to make a direct concentration determination, it can be obtained with sufficient accuracy to make this computation. (It is desired to know the concentration as accurately as possible, whereas it is usually pointless to know the relative accuracies to better than one or two significant figures.)

Table I. Optimum Scale Reading

Optical Density Scale Reading	ΔA_s for 0.2% Error in Transmittancy	$\frac{\Delta A_s}{A_s} \times 1000$ Scale Zero Set with Reagent Blank	$\frac{\Delta A_s}{A_s} \times 1000$ Scale Zero Set with Solution Having A_s Value of:			
			0.434	1.0	2.0	4.0
0.00	0.00087	∞	2.00	0.87	0.44	0.22
0.10	0.00109	10.9	2.04	0.99	0.52	0.27
0.20	0.00138	6.9	2.2	1.15	0.63	0.33
0.30	0.00173	5.8	2.4	1.33	0.75	0.40
0.434	0.00236	5.4	2.7	1.6	0.97	0.53
0.50	0.00275	5.5	2.9	1.8	1.1	0.61
0.60	0.00346	5.8	3.3	2.2	1.3	0.75
0.70	0.00435	6.2	3.8	2.6	1.6	0.93
0.80	0.0055	6.9	4.5	3.0	2.0	1.1
0.90	0.0069	7.7	5.2	3.6	2.4	1.4
1.00	0.0087	8.7	6.1	4.3	2.9	1.7
2.00	0.087	44	36	29	22	15

It is convenient to compare relative accuracies at scale readings of 0.434, as this is the optimum value utilizing the normal procedure. It is also convenient to rewrite Equation 4 in terms of the absorbency of the sample taken for the zero point in the differential procedure.

For the scale reading of 0.434 we have:

$$\frac{\text{Accuracy of differential method at 0.434}}{\text{Maximum accuracy of normal method}} = \frac{A_s \text{ for differential zero solution} + 0.434}{0.434} \tag{5}$$

OPTIMUM SCALE READING

Before proceeding further it is important to determine the optimum position in reading the optical density scale using the differential procedure. To do this it is convenient to assume that assumptions 1, 2, and 4 hold. Under these conditions the concentration error, $\Delta C/C$, can also be expressed as $\Delta A_s/A_s$ over the entire range of concentrations.

ΔA_s varies with the scale reading, but the error in determining an increment in transmittancy remains constant under any given set of conditions. Inasmuch as $\log_{10} 1/T_s = A_s$, we can compute ΔA_s from ΔT_s. Assuming a constant error of 0.2% in determining transmittancy (*1*), the values of ΔA_s corresponding to given optical density scale readings are listed in the second column of Table I. In the next column the errors in concentration resulting from various scale readings in the normal procedure are given, expressed in parts per thousand. In the succeeding columns the assumption is made that the same readings were made against differential zero solutions having A_s values of 0.434, 1.0, 2.0, and 4.0, respectively. In these cases, A_s for the solution read equals A_s for the zero solution plus the scale readings.

As can be seen from Table I, ΔA_s increases steadily with the scale reading, and is about 2.7 times as great at 0.434 as at 0.000. From this fact alone we can see that if we compared a standard and unknown solution, each having A_s values of 0.434 at zero on the scale, we would obtain 2.7 times as much accuracy as by reading the same unknown solution against a reagent blank. The concentration error in normal colorimetry reaches a minimum at 0.434 not because the scale can be read best at 0.434, but because up to this point on the scale A_s increases more rapidly than ΔA_s. As we go beyond 0.434, ΔA_s increases more rapidly than A_s.

If, however, we compare a solution to a differential zero solution having an A_s value of 0.434 or greater, the error in determining concentration will rise steadily with the scale reading, and will be lowest at 0.000. The region of nearly constant error which occurs in the normal case from about 0.3 to 0.7 does not occur with the differential solutions, and the higher the scale reading employed, the more we tend to destroy the concentration accuracy which can be gained by using the differential method. It will also be observed that the greater A_s for the differential zero solution the greater is the proportion of potentially increased accuracy which is lost by going to higher scale readings. (This point was not recognized in *2*.) The relative errors within any given vertical column in Table I depend only upon assumption 1, thus making the considerations more general.

A rigorous mathematical treatment of the complete error function in terms of transmittancies, which is highly recommended to the reader, has been made recently by Hiskey (*6*).

If A_s for the zero solution is less than 0.434 it can be shown (proceeding as in Table I) that the optimum scale reading will be at a value which makes A_s for the zero solution plus the scale reading equal to 0.434. Thus the optimum scale reading will shift from 0 to 0.434, depending on the value of A_s for the zero solution.

However, it will generally be possible with the Beckman instrument to make A_s for the zero solution greater than 0.434. If this condition is met and the unknown solution is read at zero on the scale—that is, it has the same concentration as the differential zero solution—we can write:

$$\frac{\text{Maximum accuracy of differential method}}{\text{Maximum accuracy of normal method}} = \frac{A_s \text{ for differential solution} \times 2.7}{0.434} \quad (6)$$

A_s VALUES FROM SLIT APERTURES

Whether we use Formula 5 or 6 for computing relative accuracies, we need to know A_s for the differential zero solution. It is important to remember that we do not need to know the value of this with high accuracy in making these rough computations, whereas we do need to know the increments in absorbency accurately in actually determining concentrations.

We can obtain the A_s values in most practical cases by simply reading the differential solution against a reagent blank, using the 0.1 scale when the values are above 1.0. It is desirable to be able to compute these values without making the measurements; moreover, some of the A_s values involved in the calculations exceed 3.0, the maximum which can be read directly.

These computations can be done from slit width considerations. They are necessarily approximate, but probably good enough for the purpose desired. The method is as follows:

> With the sensitivity knob set in some constant position, the slit aperture necessary to set the scale zero with a reagent blank (in many cases distilled water will do) is compared to that necessary when the solution to be used as the zero point in the differential analysis is used. In a specific case, let us assume that the above values are 0.02 and 2.0 mm., or that the second opening is 100 times the first. Actually, the slit reading applies to two slits which are operated by the same mechanism (*4*) and the light finally striking the solution passes through both of these. If both slits behave in linear fashion, it can be seen that the light striking the solution will vary as the square of the slit reading or in the case taken there will be $(100)^2$ or 10,000 times as much light striking the solution at the wider slit.
>
> From this computation T_s is evidently 1/10,000 and because $A_s = \log 1/T_s$, $A_s = 4.0$.

There are several sources of error in such a computation, such as errors in the slit mechanism, which was not intended to be used in this way (*5*). Also, in the near ultraviolet using the tungsten lamp the reference signal strength varies nonlinearly with prism rotation and a systematic error is added to the slit dial error (*5*). A very important source of error arises from the band width considerations discussed above. The calculations will hold strictly only if the color has a very broad absorption band, so that the band widths implicit in wider slit apertures do not stretch into regions of low intensity.

Table II. Comparison of Read and Calculated Values

Wave Length, mμ	A_s Read Directly	A_s Calculated from Slits	% Difference (+)
250[a]	0.691	0.724	5
270[a]	0.889	0.937	5
291[a]	0.99	1.04	5
350	1.86	1.93	4
364	2.13	2.17	2
450	1.91	2.01	5
500	1.92	2.02	5
550	1.92	1.99	4
600	2.00	2.12	6
700	1.99	2.07	4
800	2.01	2.12	5
900	2.05	2.15	5
1000	1.99	2.14	8
1100	1.95	2.12	9
		Av.	5

[a] Slit near maximum aperture.

A comparison between values read directly and computed in the above manner is given in Table II. These were obtained using appropriate solutions of potassium dichromate, potassium permanganate, nickel sulfate, and copper sulfate. Whenever possible, an A_s of about 2 was chosen, because this is about the highest value that can be read accurately on the scale, and also seems to be the most generally useful region for practical work. Below 350 millimicrons one is limited to much lower values by the fact that the slit must be normally used at wider apertures, limiting the maximum attainable A_s values.

As can be seen, the computed A_s values check reasonably well those read directly. Actually, all the calculated values are somewhat high, which would be expected on the basis of the increased band widths alone. Inasmuch as the *average* error is 5%, it would appear sensible to correct all values computed in this way by that amount. Such a correction would not have much of an effect on our computations but it would certainly improve most of the A_s values in the vicinity of 2.0. Then, too, it would partially correct for the greater discrepancies which would be expected at still higher absorbencies owing to broadening band widths. On this basis it was decided to apply a −5% correction at all wave lengths to A_s values computed from slit apertures. The value for the example computed before thus becomes 0.95 × 4.0 = 3.8.

RELATIVE ACCURACIES

It was considered desirable to compute relative accuracies using both Formulas 5 and 6, since 6 represents a maximum quantity, whereas 5 permits the use of a reasonable range in making measurements. Table III represents a series of calculations based on the assumption that a system could be found at each wave length which would obey all the restrictions on which Formulas 5 and 6 are based at maximum aperture slit. The A_s values corresponding to a 2.0-mm. slit are given at various wave lengths, computed as just described (corrected by −5%). The corresponding relative accuracies of differential and normal procedures computed from Formulas 5 and 6 are given in succeeding columns. Without altering the light source, these may be regarded as the maximum accuracy increases possible by the differential procedure on this instrument. Because of the uncertainty in computing the values, all but the smaller ones have been rounded off to the nearest whole number. The band widths of light which result from maximum aperture slits are also given in Table III.

It can be seen that the accuracy increase values fall off sharply in the ultraviolet region owing to the wider aperture slits required for normal operations. The same tendency occurs although not as markedly (in the range covered) toward the infrared end of the spectrum. The accuracy increase values in the range of 225 through at least 350 mμ are probably actually obtainable, but the very high values indicated over an appreciable portion of the spectrum probably cannot be obtained because of the great width of the spectral bands shown.

As a more reasonable basis for practical work in the spectral region above 350 mμ, Table IV has been prepared. Here the accuracy increases corresponding to 15 and 30 mμ band widths have been computed. The slit apertures shown in Table IV were computed from graphs and formulas provided with the instrument (*3*), which were also used to calculate the band widths given in Table III. The accuracy increases were computed using these slit apertures, the apertures for distilled water given in Table III, and Formulas 5 and 6:

Table III. Maximum Accuracy

Wave Length, mμ	Slit Aperture for Distilled Water[a]	A_s Corresponding to 2.0-Mm. Slit	Nominal Band Width (mμ) for 2.0-Mm. Slit	Accuracy Increase Using Formula 5	Accuracy Increase Using Formula 6
225	1.66	0.154	3.1	1.4	[b]
230	1.37	0.312	3.3	1.7	[c]
240	1.07	0.52	3.9	2.2	3.2
260	0.75	0.81	5.1	2.9	5.1
280	0.64	0.94	6.5	3.2	6
300	0.57	1.04	8.2	3.4	6
325	0.40	1.33	10.7	4.1	8
350	0.20	1.90	13.5	5.4	12
405	0.049	3.1	22	8	19
450	0.030	3.5	30	9	22
500	0.020	3.8	42	10	24
550	0.018	3.9	54	10	24
600	0.026	3.6	69	9	23
650	0.050	3.0	84	8	19
700	0.036	3.3	100	9	21
750	0.030	3.5	115	9	22
800	0.026	3.6	127	9	22
850	0.022	3.7	144	10	23
900	0.020	3.8	156	10	24
950	0.026	3.6	168	9	22
1000	0.029	3.5	177	9	22
1050	0.040	3.2	185	8	20
1100	0.072	2.7	192	7	17

Sensitivity knob set at 2 turns from clockwise end for all measurements; 1-cm. cells used throughout.

[a] Considered as pure solvent, or reagent blank.

[b,c] A_s for differential zero solution is less than 0.434, hence Formula 6 does not apply. For [b] scale reading of highest accuracy is 0.280. Reading at this point would yield 1.4 times normal accuracy. For [c] optimum scale reading is 0.122, which would yield 2.1 times normal accuracy.

The band width which can be used depends on the system under study. For potassium dichromate at 350 mμ it has been possible to use maximum aperture slit. In the case of copper (*2*) a slit corresponding to about 26 mμ was possible. Judged by the work done on these two systems and permanganate, accuracy increases of at least 5 to 6 should be possible at 0.434 and 12 to 16 at 0.0 on the scale in the spectral range of 350 to 870 mμ. [The accuracy increase of 6 computed roughly for copper in (*2*) actually corresponds to the higher range of the graph shown. It does not take into consideration the increased accuracy which should be obtained at lower scale readings.] Assuming a reproducibility of ±0.2 transmittancy the accuracy increases indicated above would correspond to concentration errors of 0.3 to 1.1 part per thousand.

The values given in Tables III and IV may vary somewhat from instrument to instrument, especially in the ultraviolet region, but should serve as working approximations. (The ultraviolet values were obtained with a hydrogen lamp which had seen considerable use; somewhat greater increase values could probably be expected with a new lamp.)

EXPERIMENTAL REPRODUCIBILITY TESTS

The preceding treatment assumes that the reproducibility in reading the scale at any given point is independent of the concentration of color used to set the zero point on the scale.

To check this reproducibility, tests were conducted by each of the authors on appropriate potassium dichromate solutions which were read at 350 mμ in 1-cm. cells. The sensitivity knob was used at about 1.5 turns from the clockwise end. Each observer was extremely careful in making the readings, but all results were obtained independently and were not compared until the measurements were completed. The results are given in Table V. The readings marked 2 in each case were obtained by replacing the solution which was being read with a fresh one, without disturbing the solution being used to set the scale zero. This was done to include errors due to wiping and positioning the cell.

The readings were not taken exactly at the same point on the scale for samples A and B but sufficiently close to permit a direct comparison of relative reproducibility. The reproducibility is about the same in both cases. The average values in each case show a maximum difference of 0.001 between first and second readings for any given observer, and a maximum difference of 0.002 between any two observers. Although the difference in concentration between zero point and sample is very nearly the same in samples A and B, the reading for A is higher than that for B. This is true in spite of the fact that (as shown in Figure 2) potassium dichromate obeys Beer's law over the higher concentration range. The effect and the reasons for it have been discussed previously. Just how serious the effect is can be ascertained as follows.

The A_s value of the differential zero solution calculated from average slits is about 1.82.

The A_s value of the 42.4054-gram standard is, therefore, about 1.82 + 0.44 = 2.26, whereas it should be about 1.82 + 0.46 = 2.28 if assumption 2 in the section "Computations of Accuracy" held. Although A_s has increased by about 500%, the

Table IV. Accuracy for 15 and 30 mμ Band Widths

Wave Length, mμ	Slit Aperture for 15 mμ Nominal Band Width	Accuracy Increase Using Formula 5	Accuracy Increase Using Formula 6	Slit Aperture for 30 mμ Band Width	Accuracy Increase Using Formula 5	Accuracy Increase Using Formula 6
405	1.37	7	17			
450	0.98	8	18			
500	0.70	8	18	1.42	9	22
550	0.54	8	18	1.10	9	21
600	0.42	6	14	0.86	8	18
650	0.34	4.6	10	0.76	6	9
700	0.28	4.9	11	0.58	6	14
750	0.24	5.0	11	0.51	6	15
800	0.21	5.0	11	0.45	6	15
850	0.19	5.1	11	0.40	7	15
900	0.17	5.1	11	0.37	7	15
950	0.16	4.5	9	0.34	6	13
1000	0.15	4.1	9	0.32	6	12
1050	0.144	3.4	7	0.31	4.9	11
1100	0.138	2.2	3.4	0.30	3.7	7

Table V. Reproducibility Tests

Sample A[a]. 8.2132 grams of 0.10000 N $K_2Cr_2O_7$ per liter read against distilled water at slit width of 0.21 to 0.22 mm.

	Observer 1		Observer 2		Observer 3	
	1	2	1	2	1	2
	0.456	0.459	0.457	0.457	0.456	0.457
	0.457	0.458	0.458	0.458	0.456	0.457
	0.457	0.457	0.457	0.458	0.456	0.457
	0.457	0.457	0.457	0.457	0.456	0.456
	0.457	0.457	0.457	0.458	0.456	0.456
			0.457	0.457	0.456	0.456
			0.457	0.458	0.456	0.455
					0.456	0.455
					0.456	0.455
					0.457	0.456
					0.456	0.456
Av. values	0.457	0.458	0.457	0.458	0.456	0.456
Difference between 1 and 2	0.001		0.001		0.000	

Maximum difference between observers, 0.002

Sample B. 42.4054 grams of 0.10000 N $K_2Cr_2O_7$ per liter read against solution containing 34.1830 grams per liter at slit width of 1.95 to 1.96 mm.

	Observer 1		Observer 2		Observer 3	
	1	2	1	2	1	2
	0.437	0.436	0.438	0.437	0.438	0.438
	0.439	0.437	0.438	0.438	0.438	0.438
	0.436	0.435	0.438	0.438	0.439	0.438
	0.437	0.436	0.438	0.437	0.438	0.439
	0.436	0.436	0.438	0.438	0.439	0.438
			0.438	0.438		
Av. values	0.437	0.436	0.438	0.438	0.438	0.438
Difference between 1 and 2	0.001		0.000		0.000	

Maximum difference between observers, 0.002

[a] A 50-ml. volumetric flask served as a weighing bottle, the solution then being transferred to the 1-liter volumetric flask. The same procedure was followed for sample B, and the same 1-liter volumetric flask was used.

loss due to increasing the slit aperture is only about 1%. This tends to justify assumption 2 at least for this case.

Errors of 0.001 to 0.002 at the indicated scale readings correspond to slightly less than errors of 0.1 to 0.2% in transmittancy. Therefore, on the basis of these tests it appears perfectly justifiable to assume that two different observers can reproduce each other with a precision of at least ±0.2% in transmittancy when the sensitivity knob is set 1.5 turns from the clockwise end.

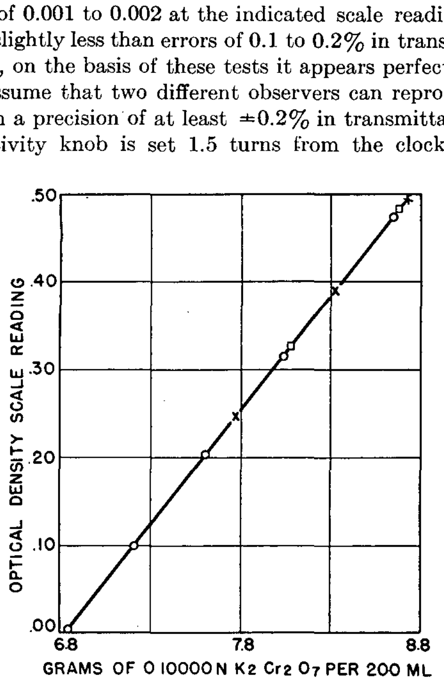

Figure 2. Analysis of Potassium Dichromate Solutions

Very nearly the same reproducibility should be obtained with the sensitivity knob at two turns from the clockwise end, the conditions under which the data in Tables III and IV were taken.

INTRODUCTION TO EXPERIMENTAL WORK

Many sources of error which may be relatively unimportant in ordinary colorimetric work must be considered in differential analysis because of the potentially increased accuracy. These include discrepancies in volumetric equipment and absorption cells, and volumetric errors due to temperature effects (the specific gravity of water varies about 2 parts in 10,000 per ° C., *7*). A very important consideration in taking advantage of the increased accuracy made possible by duplicate (or greater numbers of) determinations is that we must concern ourselves with not only the unknown solution but the differential zero solution as well. In other words, if we read three unknowns against a given zero point standard, then the standard must be located with a precision equivalent to three individual determinations as well. The failure to do this in the previous work (*2*) probably constituted a serious source of error.

Although it has been demonstrated that the most accurate results obtainable by differential colorimetry occur when the unknown is matched with the standard at zero on the optical density scale, this requires that the concentration of the "unknown" be known accurately. Although some situations may arise in which this is nearly the case, in most practical cases it is desirable to have a wider latitude in concentration.

In the work that follows the authors have used the scale from 0.0 to about 0.5. In each case the accuracy that should be obtained by reading the curve at the lowest and highest point plotted has been computed and the accuracy expressed in terms of a range. The computations have been made using $\Delta A_s/A_s$ where the A_s values have been computed from slit apertures, and the ΔA_s values have been computed assuming a reproducibility of ±0.2% transmittancy in all cases. In the case of Figures 2 and 4 this range has also been computed from Formula 1, in which S is taken directly from the experimental curve. That the same results were obtained (to the number of significant figures shown) by both methods tends to establish the validity of the slit aperture computations.

ANALYSIS OF POTASSIUM DICHROMATE SOLUTIONS

After preliminary determinations yielded the proper details, the following procedure was used:

A 0.10000 *N* and an "unknown" solution of potassium dichromate were prepared by observer 3 by direct weighing of Bureau of Standards Sample 36 which had been dried for an hour at 110° C. Both solutions were prepared in the same 2-liter volumetric flask and diluted to the mark with water at 28° C. (temperature of the water from the still and within 1° of room temperature). Observer 2 was given the known and unknown with the stipulation that the unknown be between 0.09 and 0.11 *N*. (This was the range that could be covered by the procedure to be described.)

Observer 2 obtained the data for the circles shown on Figure 2 by weighing out varying amounts of the 0.10000 *N* potassium dichromate and diluting all samples in the same 200-ml. flask with water at 28° C. These were read in a single 1-cm. absorption cell against a zero point concentration of 6.8306 grams of 0.10000 *N* potassium dichromate per 200 ml. contained in a companion cell. The zero point standard was also read against itself in the adjacent cell which provided the point near zero. The slit was used at 2.0 mm., and the sensitivity knob at 1.5 turns from the clockwise end at a wave length of 350 mμ, which is in the region of maximum absorption. An initial curve was then drawn through these points, using a 10 × 15 inch graph paper. The scale of the graph was such as to permit reading the concentration to a precision of 1 to 2 parts per 10,000 (based on the total concentration).

To obtain the additional points shown on the graph (done in batches as indicated by the different symbols), a zero standard was prepared by volume very nearly the same concentration as the original (6.84 ml. per 200 ml.). Samples were weighed and diluted as before, taking precautions only to ensure that all samples were at the same temperature and within a few degrees of 28° C. These solutions were all read in a single absorption cell against the zero point solution. The average deviation of each batch of points from the original line was determined and all of them in the group were shifted by this average amount. When all the points had been so shifted the best line was drawn through all of them.

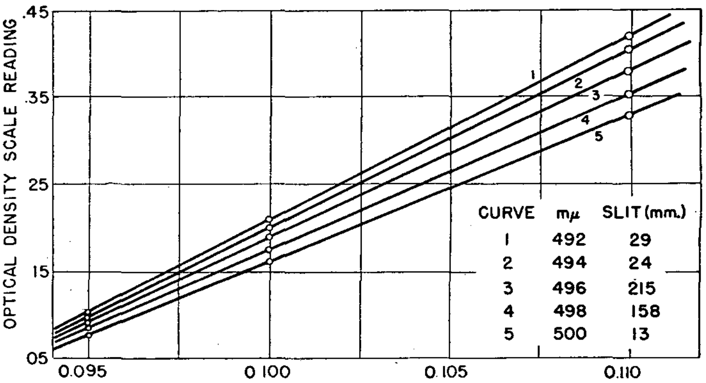

Figure 3. Direct Analysis of Potassium Dichromate Solutions

The unknown solution was allowed to stand together with the standard solution until both were at room temperature. Three standards were weighed corresponding to various regions on the graph, and three unknowns having weights in the vicinity of 7.8 grams, so as to allow for a concentration variation of about 0.09 to 0.11 *N*. All standards and unknowns were diluted in the same flask and read in the absorption cell against a zero point standard prepared as above. (Several readings were taken on each sample and the results were averaged.) The average deviation of the three standard points from the graph was determined and the unknown readings were all corrected by this value.

Unknown weights were read from the graph using the corrected scale readings and the concentration was computed from the relation:

$$\frac{\text{Weight from graph}}{\text{Actual weight of unknown}} \times 0.10000 = \text{normality of unknown}$$

Actually, the relationship as stated is not entirely correct. It would hold only if the specific gravity of the unknown and standard solutions were identical, because only then would a given weight of solution represent a given amount of solid potassium dichromate. A determination of the relative specific gravity of 0.09000 and 0.1100 *N* potassium dichromate solutions utilizing a single 50-ml. specific gravity bottle showed a difference between these of about 0.7 part per thousand. For purposes of making the small correction involved we can assume a linear relationship between specific gravity and concentration. As a matter of fact, in the case taken, no correction was needed because the concentration of the unknown was in the vicinity of the standard.

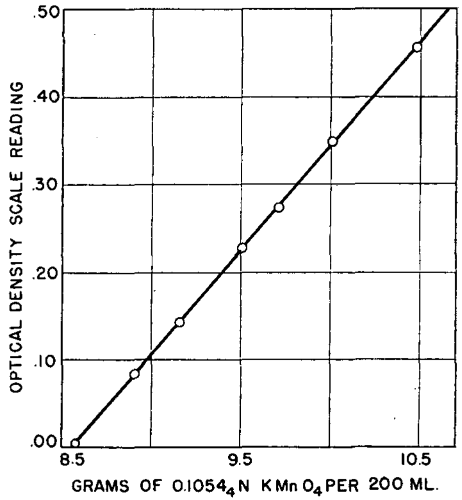

Figure 4. Analysis of Potassium Permanganate Solutions

The results obtained are given in Table VI. The accuracy is actually better than the computed range, which may indicate that the reproducibility in transmittancy is better than the assumed value. The extreme precision indicated by the average deviation probably has no special significance other than that it is better than that computed.

The above procedure is believed to meet all the difficulties described in the previous section. As long as the standards are treated exactly as the unknowns, all these sources of error tend to cancel out.

DIRECT ANALYSIS OF 0.1 *N* POTASSIUM DICHROMATE SOLUTIONS

To investigate the effect of working at higher concentrations of color, the possibility of analyzing 0.1 *N* solutions directly without further dilution was considered. This can be accomplished by working at a wave length removed from the region of maximum absorption.

Figure 3 shows a family of three-point curves at suitable wave lengths, all plotted against the same 0.09 *N* zero point. Below 492 mμ the plot was decidedly curved and consequently unsatisfactory. The 0.09893 *N* solution was checked on each curve. All solutions were at room temperature and all were read in the same absorption cell. The results are given in Table VII.

The computed accuracy range here was obtained from the least and most accurate points shown on the graphs. The precision is within the computed range, the accuracy slightly better.

Table VI. Analysis of Potassium Dichromate Solution

Normality of $K_2Cr_2O_7$ Found	Normality of $K_2Cr_2O_7$ Taken
0.09892	0.09893
0.09890	
0.09889	
Av. 0.09890 ± 0.1 part per thousand	

Computed error[a] ± 0.5 to ±1.2 parts per thousand
Error found −0.3 part per thousand

[a] Based on scale readings of 0.00 and 0.50 and ±0.2% transmittancy.

Table VII. Analysis of 0.09893 *N* Potassium Dichromate Solution

Wave Length, mμ	Normality Found
500	0.09890
498	0.09888
496	0.09896
494	0.09901
492	0.09910
	Av. 0.09897 ± 0.7 part per thousand

Computed error ±0.5 to ±1.0 part per thousand
Error found +0.4 part per thousand

In working away from the region of maximum absorption, however, great care must be taken in displacing and resetting the wave-length scale. This is due to the fact that we are in a region where the absorption curve is steeper than at the point of maximum absorption (*8*). In the above case, a magnifier was placed over the wave-length scale and two lines were drawn on the glass equidistant from the hair line to prevent errors due to parallax. The wave-length scale was displaced and reset several times in the course of the analysis.

ANALYSIS OF POTASSIUM PERMANGANATE SOLUTIONS

Two potassium permanganate solutions were prepared and standardized under as nearly identical conditions as possible by observer 3, utilizing Bureau of Standards sodium oxalate 40e and the procedure recommended by the bureau (*10*). Because both samples were titrated under nearly identical conditions, no blank correction was applied in either case.

Colorimetric work was done by observer 2 in a manner similar to that already described. The work was done at 526 mμ, which is in the region of maximum absorption for potassium permanganate, slit width 0.25 mm., sensitivity knob setting 1.5 turns from clockwise end. Figure 4 and Table VIII summarize the results obtained. The specific gravity correction was made on the basis of a direct comparison of the known and unknown solutions in the "50-ml." specific gravity bottle.

Table VIII. Normality of Potassium Permanganate Solutions

	Found (Colorimetric)	Found (Oxalate)
	0.09865	0.09898
	0.09888	0.09888
	0.09882	0.09886
	0.09885	0.09897
		0.09890
		0.09886
Av.	0.09880 ± 0.8	0.09891 ± 0.5 part per thousand
Value corrected for specific gravity	0.09878	

Computed error ±0.4 to ±1.0 part per thousand
Error found (taking oxalate value as correct) −1.3 parts per thousand
Based on scale readings of 0.00 and 0.46 and precision of ±0.2% in transmittancy.

The results of the oxalate standardization on the permanganate from which Figure 4 was prepared showed an average deviation of ±0.7 part per thousand. In view of this, the reader might question expressing the results shown to a precision of 0.1 part per thousand. This has been done only to compare the precision of the colorimetric and volumetric methods more closely. Actually, the average colorimetric value is very nearly within the experimental error involved in the oxalate standardizations.

DISCUSSION

A very inviting possible application of differential colorimetry would be in the determination of a base metal in an alloy, the content of which was known to be close to 100%, or in the analysis of a "pure" metal. Here a very short range graph could be prepared in the vicinity of zero on the optical density scale. It might also be possible to obtain an accuracy of 0.1% transmittancy over such a short range. All these factors would make for extreme accuracy.

CONCLUSION

Differential colorimetry with the present Model DU Beckman spectrophotometer is capable of yielding results as accurate as most gravimetric and volumetric methods of analysis. The problem of finding colors that are stable and reproducible enough to utilize needs investigation.

ACKNOWLEDGMENT

The authors wish to thank Joan Stewart and Frank Bassani for preparing the graphs shown in this paper.

ADDENDA

Some time after the data in Table III were taken, the ultraviolet source employed burned out. The lamp, together with its reflector which showed signs of wear, was replaced and the ultraviolet data were rechecked at a sensitivity equivalent to that previously employed. The results are given in Table IX.

Table IX. Ultraviolet Accuracy Increase in Values with New Lamp

Wave Length, mμ	Slit Aperture for Distilled Water	A_s Corresponding to 2.0-Mm. Slit	Accuracy Increase Using Formula 5	Accuracy Increase Using Formula 6
225	0.50	1.14	3.6	7
240	0.41	1.31	4.0	8
260	0.36	1.42	4.3	9
280	0.34	1.46	4.4	9
300	0.33	1.49	4.4	9

As anticipated, the accuracy increase values are greater than those given in Table III, the most appreciable differences being at wave lengths below 260 mμ. Based on these results, differential colorimetry would be feasible even at the shorter wave lengths. The data given should merely be taken as indicative of the performance to be expected from a new lamp, because these lamps probably vary in their light output.

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Chromium and Manganese in Steel and Ferroalloys

Simultaneous Spectrophotometric Determination

JAMES J. LINGANE and JUSTIN W. COLLAT
Harvard University, Cambridge 38, Mass.

The spectrophotometric method of Silverthorn and Curtis has been re-examined to determine whether the empirical calibration with standard steel samples is necessary. A study of the various optical and chemical factors demonstrates that Beer's law is obeyed and the manganese and chromium contents can be computed from the densities observed at two appropriately chosen wave lengths. Corrections have been established for the effect of other elements commonly present in ferroalloys. Results obtained are equal in precision and accuracy to values derived from empirical calibrations.

SILVERTHORN and Curtis (*5*) described a spectrophotometric method for the simultaneous determination of chromium and manganese in steel, which inherently is of great practical utility. The method is based on a persulfate oxidation of the two elements to dichromate ion and permanganate ion, in the presence of phosphoric acid to decolorize the ferric iron. From measurements of the optical density of the solution at two appropriate wave lengths the chromium and manganese contents can be computed.

Under the particular conditions that they selected, Silverthorn and Curtis found that it was necessary to calibrate the method empirically with a series of standard steel samples. It is not clear from their description whether this apparent failure of Beer's law was due to the chemical aspects of their procedure or whether it was instrumental in nature and a reflection of the spectrophotometric technique which they employed.

The purpose of the present investigation was a critical study of the various aspects of the Silverthorn and Curtis method. It was found that under the proper chemical conditions, and when the absorption measurements are made with a spectrophotometer that operates with a relatively narrow spectral band width, Beer's law is obeyed and it is not necessary to resort to empirical calibration. In the improved procedure periodate ion is used in addition to persulfate ion to ensure complete oxidation of the manganese and to eliminate the fading of the permanganate which occurs when persulfate is used alone. The influence of other elements commonly present in steels and ferroalloys has also been investigated and the appropriate corrections have been established.

EXPERIMENTAL TECHNIQUE

Absorption spectra were measured from 220 to 1100 $m\mu$ with a Beckman Model DU quartz spectrophotometer, using 1-cm. quartz cells which had been matched against each other. (The less expensive Corex glass cells may be used in the actual analyses, inasmuch as measurements do not need to be made below 440 $m\mu$.) The slit widths were adjusted so that the spectral band widths were smaller than 5 $m\mu$ at all wave lengths, and in the range from 300 to 700 $m\mu$ it was usually possible to employ a band width as small as 1 $m\mu$ when care was taken to maintain the optical system in optimum focus.

The absorption spectra shown in Figure 1 were measured against the same concentrations of sulfuric and phosphoric acids in the reference cell. Later it was found that a sulfuric-phosphoric acid solution does not absorb over the range of wave lengths used in the analyses, and hence water may be used in the reference cell.

A standard 1 millimolar solution of +2 manganese was prepared determinately from pure manganous oxalate dihydrate (*3*), by decomposing a 179.0-mg. sample of the salt in 5 ml. of hot concentrated sulfuric acid and finally diluting to 1 liter.

Standard dichromate solutions were prepared determinately from pure potassium dichromate. Standard solutions of pervanadyl ion—nickel, cobalt, copper, and iron—were prepared from purified ammonium vanadate, nickel ammonium sulfate hexahydrate, cobaltous sulfate heptahydrate, cupric sulfate pentahydrate, and Bureau of Standards ingot iron. All reagents were of analytical reagent quality.

Measured volumes of these solutions were carried through the oxidation procedure described below to obtain the solutions used for the determination of the extinction coefficients.

RESULTS AND DISCUSSION

Absorption Spectra. Judged by the absorption spectra in Figure 1, an optimum wave length for the measurement of pure dichromate solutions would be at the peak in the near ultraviolet at 350 $m\mu$. However, even in the presence of phosphoric acid, ferric iron begins to absorb so strongly below about 425 $m\mu$ that shorter wave lengths than this are inaccessible in steel analyses, and it is necessary to employ the weaker broad band at 440 $m\mu$. Even at 440 $m\mu$ the correction for ferric iron absorption corresponds to 0.0005% chromium for each 1% of iron, and hence amounts to 0.03 to 0.05% chromium with most steels.

From the curves for dichromate and permanganate in the region 400 to 450 $m\mu$ it is seen that 425 $m\mu$ at the minimum in the permanganate curve would be the optimum wave length for measurement of the mixture, for at this point the ratio of the extinction coefficients of dichromate and permanganate is maximal. However, because the correction for iron adsorption is greater at 425 than at 440 $m\mu$, measurement at the latter wave length is the best compromise.

Silverthorn and Curtis (*5*) measured the permanganate extinction at 575 $m\mu$ and at this wave length the extinction due to dichromate ion is negligibly small. The present authors prefer to measure at the absorption maximum at 545 $m\mu$, which provides greater precision, and to apply the small correction for dichromate absorption. Because the apparent extinction coefficient at the sharp absorption maximum decreases with increasing band width, the analytical measurements must be made with the same slit width used to determine the extinction coefficient. The apparent extinction coefficient at 545 $m\mu$ decreased by 2.4% when the slit width was increased from 0.01 to 0.2 mm., corresponding to an increase in the band width from 0.8 to 5.3 $m\mu$. At 575 $m\mu$ an increase in band width from 0.9 to 3.1 $m\mu$ caused the apparent extinction coefficient to increase by 3.9%. At 440 $m\mu$ the apparent extinction is, of course, much less sensitive to band width.

Validity of Beer's Law for Dichromate. The fact that potassium dichromate solutions do not obey Beer's law in very dilute (0.005 *N*) sulfuric acid, but show an increasing extinction coefficient with increasing concentration, is well known from the excellent study by Kortüm (*2*). This apparent deviation at relatively small hydrogen ion concentrations is doubtless partly due to the shift of the chromate ion–dichromate ion equilibrium with changing total chromium concentration.

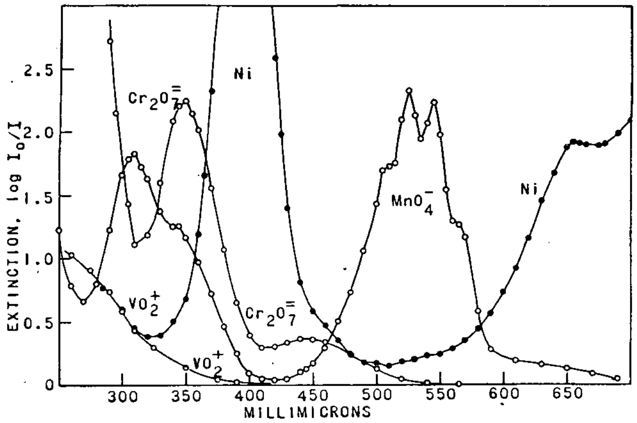

Figure 1. Absorption Spectra

1 millimolar permanganate ion, dichromate ion, and pervanadyl ion, and 100 millimolar nickelous ion, in 1 *M* sulfuric acid–0.7 *M* phosphoric acid

In solutions of high hydrogen ion concentration Beer's law is accurately obeyed, as shown by the data in Table I. In this table, *D* is the observed optical density (extinction), defined by $D = \log(I_0/I) = \epsilon l C$, where ϵ is the extinction coefficient in the units cm.^{-1} (millimoles per liter)$^{-1}$, l is the cell length (cm.), and C is concentration (millimoles per liter). The value of ϵ is constant to ±0.5% over the concentration range which can be measured with a 1-cm. cell.

The extinction coefficient of dichromate ion decreases with increasing concentration of sulfuric acid, but above 0.5 *M* acid the rate of change is not very great—for example, with 1.667 millimolar dichromate ion in 0.5, 1.0, 2.0, and 3.0 *M* sulfuric acid the observed densities at 440 $m\mu$ were seriatim 0.611, 0.610, 0.604, and 0.597.

Multicomponent determinations rest on the assumption that the substances concerned contribute independently to the total optical density at a given wave length. This was verified for the

Table I. Extinction Coefficient of Dichromate Ion

(Measured at 440 $m\mu$ in a 1-cm. cell with a band width of 0.5 $m\mu$. Solutions were 1 *M* in sulfuric acid and 0.7 *M* in phosphoric acid.)

$K_2Cr_2O_7$ Millimolar	*D*	ϵ
0.166	0.062	0.373
0.417	0.153	0.367
0.833	0.308	0.370
1.250	0.459	0.367
1.662	0.609	0.367
4.17	1.544	0.370
		Av. 0.369 ± 0.002

Table II. Test of Additivity Principle with Dichromate-Permanganate Mixtures at 440 $m\mu$

(Measured density of the dichromate solution alone was 0.627 and that of the permanganate solution was 0.376. Both solutions were 1 *M* in sulfuric acid.)

Dichromate Soln., Ml.	Permanganate Soln., Ml.	*D* Obsd.	*D* Calcd.
50	5	0.607	0.604
25	5	0.588	0.585
25	10	0.558	0.555
25	25	0.502	0.502
10	25	0.448	0.448
5	50	0.397	0.399

present case by preparing separate solutions of dichromate ion and permanganate ion and measuring the density of each at 440 mμ. The solutions were then mixed in various ratios from 0.1 to 10 and the optical density of each mixture was measured at 440 mμ. The data in Table II demonstrate that the observed densities of the mixture agree with those calculated from the individual densities and the mixing ratio, and hence the additivity principle is valid in this case.

Calculational Technique. Assuming for the moment that absorption at 440 and 545 mμ is due only to dichromate and permanganate ions, the following relations are valid for a 1-cm. light path:

$$D_{440} = {}_{440}\epsilon_{\mathrm{Cr}}\, C_{\mathrm{Cr}} + {}_{440}\epsilon_{\mathrm{Mn}}\, C_{\mathrm{Mn}} \tag{1}$$

$$D_{545} = {}_{545}\epsilon_{\mathrm{Cr}}\, C_{\mathrm{Cr}} + {}_{545}\epsilon_{\mathrm{Mn}}\, C_{\mathrm{Mn}} \tag{2}$$

where the subscripts Cr and Mn denote dichromate ion and permanganate ion, and concentrations are expressed in millimoles per liter. Simultaneous solution of these two equations yields

$$C_{\mathrm{Mn}} = \frac{{}_{440}\epsilon_{\mathrm{Cr}}\, D_{545} - {}_{545}\epsilon_{\mathrm{Cr}}\, D_{440}}{{}_{440}\epsilon_{\mathrm{Cr}}\; {}_{545}\epsilon_{\mathrm{Mn}} - {}_{545}\epsilon_{\mathrm{Cr}}\; {}_{440}\epsilon_{\mathrm{Mn}}} \tag{3}$$

When the values of the various extinction coefficients (Tables III and IV) are introduced, Equation 3 becomes

$$C_{\mathrm{Mn}} = 0.426\, D_{545} - 0.013\, D_{440} \tag{4}$$

Combining Equation 4 with Equation 1 yields for the concentration of dichromate ion

$$C_{\mathrm{Cr}} = 2.71\, D_{440} - 0.110\, D_{545} \tag{5}$$

Correspondingly, the percentages of manganese and chromium in a sample of W grams in a volume of V ml. are

$$\text{Manganese, \%} = \frac{0.00549\, V}{W}(0.426\, D_{545} - 0.013\, D_{440}) \tag{6}$$

$$\text{Chromium, \%} = \frac{0.01040\, V}{W}(2.71\, D_{440} - 0.110\, D_{545}) \tag{7}$$

where the constants 0.00549 and 0.01040 are 10^{-4} multiplied by the atomic weight of manganese and twice the atomic weight of chromium, respectively.

Of the other elements commonly present in ferroalloys, vanadium, cobalt, and nickel, exhibit extinction coefficients at these wave lengths which are sufficiently large so that corrections must be subtracted from the apparent manganese and chromium percentages computed from the foregoing equations when moderately large amounts of these elements are present. Correction for iron itself is also appreciable at 440 mμ.

The absorption spectra of pervanadyl ion and nickelous ion are shown in Figure 1. The nickel spectrum corresponds to a concentration 100 times larger than the other elements. Absorption by pervanadyl ion is still appreciable at 440 mμ but negligible at 545 mμ. Absorption by nickelous ion is also more significant at 440 than at 545 mμ. The spectrum of cobaltous ion (not shown in Figure 1) comprises a broad band with a maximum at 510 mμ.

When the percentages of nickel, cobalt, and vanadium are known, the corresponding corrections are most conveniently made in terms of the optically equivalent chromium and manganese percentages computed once and for all from the experimentally determined extinction coefficients. These corrections are listed in Tables III and IV, as the equivalent chromium or manganese percentages corresponding to each 1% of the element in question which must be subtracted from the apparent chromium and manganese percentages computed by Equations 6 and 7 to obtain the true values. Expressed in this way, these corrections are independent of the sample weight. In all cases the corrections on the chromium are much larger than on the manganese, which tends to render the chromium determination somewhat less precise than that of the manganese.

Table III. Chromium Corrections at 440 mμ

(Values listed under Correction are equivalent percentages of chromium to be subtracted from apparent chromium percentage for each 1% of element in question.)

Substance	ε	Correction, %
$Cr_2O_7^{--}$	0.369	.....
MnO_4^-	0.095	0.490
VO_2^+	0.00482	0.0266
Co^{++}	0.0015	0.0072
Ni^{++}	0.0008	0.0039
Fe^{+++}	0.0001	0.0005

Table IV. Manganese Corrections at 545 mμ

(Values listed under Correction are equivalent manganese percentages to be subtracted from apparent manganese percentage for each 1% of the element in question.)

Substance	ε	Correction, %
MnO_4^-	2.35	.....
$Cr_2O_7^{--}$	0.011	0.0025
Co^{++}	0.0027	0.0011
Ni^{++}	0.0002	0.0001
VO_2^+	Negligible	.....
Fe^{+++}	Negligible	.....

Ewing and Parsons (*1*) recently demonstrated that apparent extinction coefficients measured with ten different Beckman Model DU spectrophotometers differed slightly but significantly from each other, even though the several instruments and measurement conditions were apparently identical. The differences are small enough so that the corrections for nickel, cobalt, vanadium, and iron listed in Tables III and IV may safely be used with different Beckman Model DU instruments, and probably they may also be used with the simplified Model B Beckman spectrophotometer. It is advisable, however, to determine the extinction coefficients of dichromate ion and permanganate ion at 440 and 545 mμ with the particular instrument used, employing solutions of known concentrations of dichromate ion and permanganate ion prepared according to the procedure described in the following section.

Procedure. Persulfate (peroxydisulfate) ion in the presence of silver ion as a catalyst is one of the few oxidants which quantitatively oxidize both chromic ion and manganous ion to dichromate ion and permanganate ion in acid solution and it has long been a favored reagent for this purpose (*4*). The oxidation may be performed in solutions containing (either or both) sulfuric and nitric acids. The optimum acidity is stated (*4*) to be between 0.5 and 1.5 molar sulfuric acid; with too low an acid concentration manganese dioxide tends to precipitate, and the oxidation of chromium is inhibited at greater acidities.

Although the persulfate oxidation of chromium is invariably satisfactory, oxidation of manganese occasionally is erratic and incomplete. In the hot acid solution required persulfate ion decomposes rapidly by oxidizing water, $2S_2O_8^{--} + 2H_2O = O_2 +$

$4SO_4^{--} + 4H^+$, and various substances can catalyze this reaction at the expense of the manganous ion–persulfate ion reaction.

Furthermore, permanganate solutions prepared by a persulfate oxidation are not stable, but fade rather rapidly, and hence the measurements must be made immediately after the oxidation. In a typical instance the optical density at 545 mμ of a permanganate solution prepared by persulfate oxidation in 1 *M* sulfuric acid and 0.3 *M* phosphoric acid decreased by 2.4% per hour.

These difficulties are eliminated by the combined use of persulfate ion and periodate ion. The superiority of periodate ion for the oxidation of manganese was originally demonstrated by Willard and Greathouse (*6*). Permanganate solutions prepared by periodate oxidation are stable indefinitely. Because periodate ion does not oxidize chromium quantitatively, persulfate ion is retained for this purpose in the present procedure.

By appropriate choice of sample weight and final volume of the oxidized solution the following procedure may be applied to steels, ferroalloys, and copper-nickel alloys with manganese contents between about 0.1 and 5% and chromium contents from a few tenths of 1% up to 20% or more. The sample weight and final volume should be chosen so that the optical densities at 440 and 545 mμ fall in the range 0.1 to about 1.5.

For an excellent discussion of various methods of dissolving chromium steels the monograph of Lundell, Hoffman, and Bright (*4*) should be consulted. In most cases solution can be effected in hot, dilute sulfuric acid, a mixture of sulfuric and nitric acids, or concentrated perchloric acid. When tungsten is present phosphoric acid is added to the dissolving acid to prevent precipitation of tungstic oxide. When much carbonaceous residue is left the solution must be evaporated to sulfuric acid fumes to obtain complete decomposition of chromium carbide.

Obviously, the final solution must be optically clear, and if there is any turbidity in the sample solution the aliquot taken for the oxidation must be filtered or centrifuged.

Table V. Test of Method with Bureau of Standards Samples

	Sample and N.B.S. Certificate Values	% Found	
		Cr	Mn
1.	Mn steel 100 Cr 0.18, Mn 1.38, Ni 0.151, V 0.011, Cu 0.124, C 0.617	0.23	1.37
2.	Nickel-copper alloy 162 Cr 0.24, Mn 2.34, Fe 0.34, Ni 66.38, Cu 28.93, Co 0.54, Ti 0.20, Al 0.23, Si 0.67, C 0.11	0.21	2.33
3.	Ni-Mo steel 111A Cr 0.242, Mn 0.743, Ni 1.75, Mo 0.222, V 0.002, Cu 0.079, C 0.203	0.24	0.737
4.	Steel 32C Cr 0.654, Mn 0.752, Ni 1.20, V 0.003, Mo 0.063, Cu 0.099, C 0.429	0.69	0.741
5.	Ferrovanadium 61A Cr 0.68, Mn 1.78, V 50.2, Si 5.11, Fe 41.0	0.77	1.77
6.	Cr-V steel 30d Cr 1.15, Mn 0.786, Ni 0.150, V 0.190, Mo 0.034, Cu 0.092, C 0.363	1.16	0.781
7.	Cu-Ni-Cr cast iron 115 Cr 2.17, Mn 1.01, Ni 15.89, Cu 6.44, C 2.42	2.17	0.99
8.	Ni-W-Cr-V steel 134 Cr 3.73, Mn 0.155, Mo 8.68, W 1.82, V 1.13, Ni 0.077, C 0.810	3.69	0.155
9.	Mo-W-Cr-V steel 132 Cr 4.11, Mn 0.252, Ni 0.094, V 1.64, Mo 7.07, W 6.29, C 0.803	4.05	0.250
10.	Co-Mo-W steel 153 Cr 4.14, Mn 0.22, V 2.03, Co 8.43, Ni 0.12, W 1.58, Mo 8.36, Cu 0.10, C 0.86	4.08	0.204
11.	Steel 135 Cr 5.15, Mn 0.458, Ni 0.083, V 0.010, Mo 0.575, Cu 0.076, C 0.094	5.14	0.459
12.	18 Cr-8Ni steel 101b Cr 18.49, Mn 0.597, Ni 8.99, V 0.049, Mo 0.078, Co 0.078, Cu 0.168, C 0.069	18.4	0.575
13.	18 Cr-10 Ni steel 121 A Cr 18.69, Mn 1.28, Ni 10.58, V 0.035, Mo 0.020, Co 0.090, Ti 0.361, C 0.073	18.6	1.27

To a 1-gram sample in a 300-ml. Kjeldahl flask add 30 ml. of water and 10 ml. of concentrated sulfuric acid (also 10 ml. of 85% phosphoric acid if tungsten is present). Boil gently until decomposition is complete or the reaction subsides. Then add 5 ml. of concentrated nitric acid in several small portions. If much carbonaceous residue persists, add 5 ml. more of nitric acid, and boil down to copious fumes of sulfuric acid. Dilute to about 100 ml. and boil until all salts have dissolved. Cool, transfer to a 250-ml. volumetric flask, and dilute to the mark.

Pipet a 25- or 50-ml. aliquot of the sample solution (centrifuged or filtered if not perfectly clear) into a 250-ml. Erlenmeyer flask. Add 5 ml. of concentrated sulfuric acid, 5 ml. of 85% phosphoric acid, and 1 to 2 ml. of 0.1 *N* silver nitrate solution, and dilute to about 80 ml. Add 5 grams of pure potassium persulfate, swirl the flask until most of the salt has dissolved, and heat to boiling. Keep at the boiling point for 5 to 7 minutes. Cool slightly, and add 0.5 gram of pure potassium periodate. Again heat to boiling and keep at the boiling point for about 5 minutes. Cool, transfer to a 100-ml. volumetric flask, and dilute to the mark. Transfer a portion of the solution to a 1-cm. absorption cell and measure the optical densities at 440 and 545 mμ. Compute the percentages of chromium and manganese as described above.

Results. The validity of the foregoing procedure is substantiated by the results shown in Table V, obtained with a wide variety of National Bureau of Standards steel samples, a sample of ferrovanadium, and a nickel-copper alloy. All the elements likely to be encountered in the analysis of ferroalloys are represented by these samples. These results are based on the direct application of Beer's law, using the extinction coefficients obtained with pure dichromate and permanganate solutions.

The fact that satisfactory chromium results were obtained with the low-chromium steels, with the ferrovanadium, and with the nickel-copper alloy is especially noteworthy, because in these unfavorable cases the total corrections due to manganese, vanadium, and nickel are much larger than the small chromium contents.

SUMMARY

An improved and rapid method for the simultaneous spectrophotometric determination of chromium and manganese, based on the measurement of the cumulative optical densities due to dichromate ion and permanganate ion at 440 and 545 mμ, is described. When measurements are made with a spectrophotometer employing a narrow spectral band width (5 mμ or smaller), and with solutions containing at least 0.5 *M* sulfuric acid, absorption by dichromate ion, permanganate ion, and mixtures of the two strictly follows Beer's law.

Ferric iron (even in the presence of phosphoric acid), nickel, cobalt, and vanadium absorb appreciably at 440 and 545 mμ, and the appropriate corrections have been established.

The oxidation of the chromium and manganese in a solution of a steel is best performed by the combined use of peroxydisulfate ion and periodate ion.

Very satisfactory results were obtained with many different types of Bureau of Standards steels and ferroalloys, ferrovanadium, and copper-nickel alloys containing from 0.1 to 5% manganese and 0.2 to 20% or more chromium. None of the elements commonly present in ferroalloys interferes.

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Rapid Photometric Determination of Copper in Ferrous Alloys

Use of Alpha-Benzoinoxime

RAYMOND A. DUNLEAVY[1], STEPHEN E. WIBERLEY, AND JOHN H. HARLEY[2]
Rensselaer Polytechnic Institute, Troy, N. Y.

A direct colorimetric method for the determination of copper in plain and low-alloy steels and cast irons has been developed. A nitric acid solution of the steel is treated with Rochelle salt to complex iron and the pH is adjusted to 11.3 to 12.3 with sodium hydroxide. A solution of α-benzoinoxime in sodium hydroxide is added and the green copper complex is extracted with chloroform. The color may be measured with a spectrophotometer or a filter photometer. The method is rapid and accurate. Three samples may be run in about 20 minutes, and the average error of a single determination is 0.005% copper. The only elements causing appreciable interference are nickel (above 2%) and cobalt (above 0.25%).

AT THE present time, there is need for a simple rapid method for the determination of the small amounts of copper present in cast irons and steels. The reagent α-benzoinoxime is relatively specific for copper and has been used as a gravimetric reagent. The present investigation has been directed towards determining the proper conditions for the use of α-benzoinoxime in the colorimetric determination of copper in iron and steel.

Feigl (*1*) reported the use of α-benzoinoxime as a reagent for copper in 1924. Kar (*3*) and Silverman (*4*) reported its use for the gravimetric determination of copper in steels. Although Feigl had stated previously that α-benzoinoxime was specific for copper, Jennings (*2*) reported that nickel, cobalt, platinum, and palladium also formed complexes with the reagent.

REAGENTS AND EQUIPMENT

Alpha-benzoinoxime (Eastman Kodak), 0.5% solution in 0.25 *N* sodium hydroxide.
Sodium hydroxide, 10% in water.
Rochelle salt, 300 grams in 500 ml. of water.
Chloroform, reagent grade.
Beckman spectrophotometer, Model DU.
Klett-Summerson filter photometer, with test-tube attachment.
Beckman pH meter, Model M.

PROCEDURE FOR DETERMINATION OF COPPER

In Steel. Weigh a 0.500-gram sample into a 150-ml. beaker and dissolve in 20 ml. of 1 to 2 nitric acid. If necessary, add a drop or two of 30% hydrogen peroxide to dissolve any manganese dioxide and boil off the oxides of nitrogen. Cool slightly, and add 25 ml. of Rochelle salt solution and 30 ml. of 10% sodium hydroxide. Adjust the pH to the range 11.3 to 12.3 with 10% sodium hydroxide and add 2 ml. (pipet) of 0.5% α-benzoinoxime solution.

Transfer to a 250-ml. pear-shaped separatory funnel, add 40 ml. of chloroform, and shake vigorously for three 30-second periods. Filter the chloroform through a small paper into a 50-ml. volumetric flask, and rinse by running 5 ml. of chloroform through the separatory funnel and the filter. Make up the extract to volume with chloroform and measure the intensity of the color. (The maximum absorbency is at 440 mμ, and measurements with the spectrophotometer were made at this wave length. Measurements with the filter photometer were made with a combination filter peaked at 430 mμ.)

In Cast Irons. Inasmuch as the large amounts of graphitic carbon and silica present in cast iron collect at the interface, it has not been found necessary to remove them by filtration. The above procedure may be used without change.

If the sample does not dissolve readily in the 1 to 2 nitric acid, a few drops of hydrochloric acid may be used to complete solution.

The calibration curve was obtained by adding known amounts of a standard copper solution to 0.5-gram samples of Bureau of Standards steel 22b. With the spectrophotometer, the curve was linear up to at least 0.21% copper, while with the filter photometer the curve tapered off above 0.15% copper.

ANALYTICAL RESULTS

A group of Bureau of Standards steels and cast irons of various compositions were run using the above method. Table I contains all the results obtained, which represent accidental errors as well as those inherent in the method.

For 28 steels and irons, the average error for a single determination is 0.0045% copper with the spectrophotometer, and 0.005% copper with the filter photometer. The corresponding errors for the average of triplicates are 0.0035 and 0.0045% copper.

EFFECT OF VARIABLES

Chloroform was found to be the most suitable solvent for the copper compound on the basis of high solubility and color stability. Chloroform, alcohol, acetone, ammonium hydroxide, and sodium hydroxide were tried as solvents for the reagent itself. Both chloroform and ammonium hydroxide gave poor extraction, alcohol and acetone caused rapid fading of the color, but sodium hydroxide was entirely satisfactory.

Effect of pH. Solutions of steel containing the equivalent of 0.106% copper were adjusted to various pH values with 10%

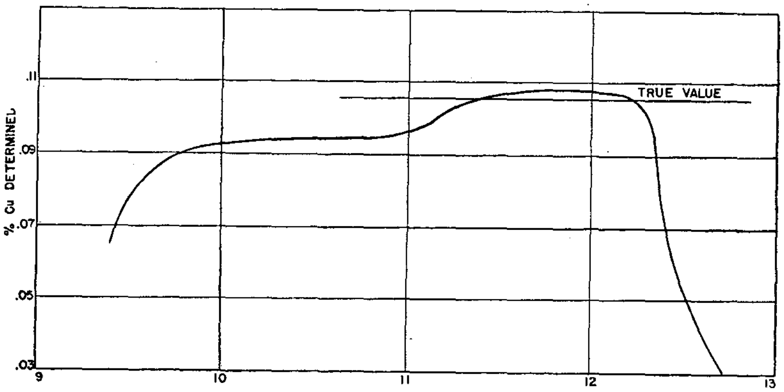

Figure 1. Effect of pH on Extraction of Copper

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[1] Present address, Department of Chemistry, University of Cincinnati, Cincinnati, Ohio.

[2] Present address, U. S. Atomic Energy Commission, New York Operations Office, New York, N. Y.

Table I. Determination of Steel and Cast Iron

B. S. Steel	Beckman, % 1	Beckman, % 2	Beckman, % 3	Beckman, % Av.	Klett-Summerson, % 1	Klett-Summerson, % 2	Klett-Summerson, % 3	Klett-Summerson, % Av.	True Value, %	Operator
Bessemer										
8c	0.008	0.008	0.008	0.008	0.008	0.008	0.007	0.008	0.010	2
8c	0.006	0.005	0.004	0.005	0.005	0.004	0.004	0.004	0.010	1
9c	0.016	0.016	0.012	0.014	0.015	0.015	0.015	0.015	0.020	1
10c	0.018	0.017	0.017	0.017	0.015	0.015	0.015	0.015	0.020	1
22b	0.008	0.007	0.007	0.007	0.008	0.008	0.007	0.008	0.009	2
23a	0.008	0.007	0.010	0.008	0.008	0.007	0.010	0.008	0.014	1
B.O.H. steel										
11b	0.014	0.013	0.012	0.013	0.012	0.012	0.012	0.012	0.014	3
14b	0.012	0.014	0.018	0.015	0.012	0.013	0.014	0.013	0.017	1
15c	0.138	0.138	0.136	0.137	0.126	0.126	0.126	0.126	0.141	1
16c	0.050	0.062	0.072	0.061	0.048	0.066	0.068	0.061	0.060	1
A.O.H. steel										
19c	0.162	0.165	0.164	0.164	0.150	0.150	0.150	0.150	0.161	3
20d	0.155	0.160	0.150	0.155	0.156	0.156	0.154	0.155	0.164	3
21c	0.046	0.056	0.052	0.051	0.048	0.056	0.052	0.052	0.050	1
34	0.016	0.012	0.012	0.013	0.014	0.012	0.012	0.013	0.015	1
35	0.054	0.052	0.052	0.053	0.054	0.052	0.052	0.053	0.06	1
Cast iron										
4e	0.012	0.012	0.013	0.012	0.012	0.011	0.012	0.012	0.010	1
6d	0.150	0.166	0.142	0.153	0.148	0.154	0.138	0.147	0.151	1
82	0.021	0.024	0.021	0.022	0.026	0.026	0.026	0.026	0.020	1
107	0.054	0.062	0.080	0.066	0.064	0.065	0.080	0.069	0.074	1
122a	0.044	0.045	0.048	0.046	0.046	0.046	0.048	0.047	0.045	1
Low-alloy steel										
24	0.022	0.024	0.025	0.024	0.022	0.022	0.022	0.022	0.022	1
33a	0.064	0.072	0.064	0.068	0.070	0.076	0.070	0.072	0.080	1
36	0.110	0.116	0.116	0.114	0.110	0.118	0.118	0.115	0.112	3
72a	0.082	0.084	0.084	0.083	0.082	0.083	0.082	0.082	0.079	1
111	0.122	0.130	0.114	0.122	0.124	0.132	0.120	0.125	0.122	1
Electric steel										
51	0.044	0.036	0.038	0.039	0.043	0.034	0.034	0.037	0.044	1
65	0.060	0.059	0.059	0.059	0.065	0.062	0.058	0.062	0.060	2
Ingot iron										
55b	0.043	0.041	0.036	0.040	0.042	0.041	0.038	0.040	0.042	3
Rail steel										
100	0.127	0.128	0.128	0.128	0.124	0.124	0.124	0.124	0.124	1

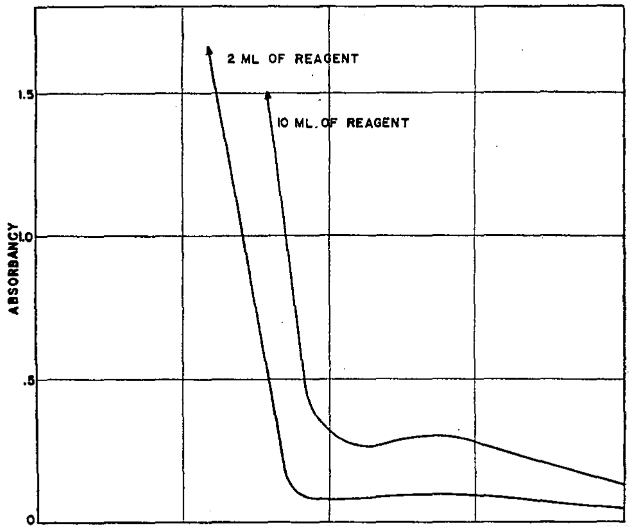

Figure 2. Absorbency of Nickel-α-Benzoinoxime

5 mg., equivalent to 1% nickel, present

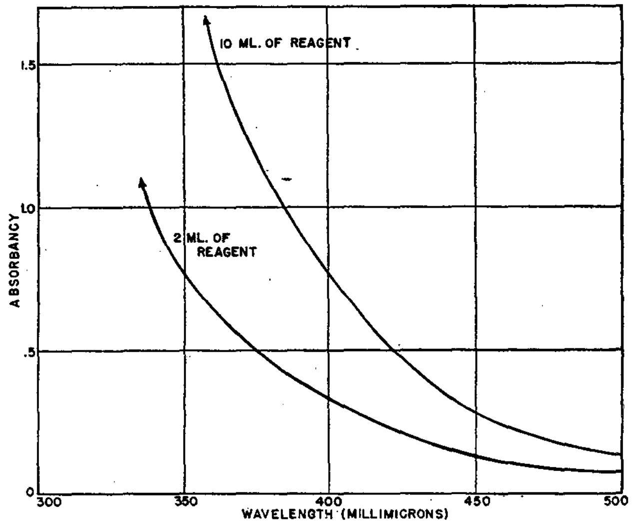

Figure 3. Absorbency of Cobalt-α-Benzoinoxime

5 mg., equivalent to 1% cobalt, present

sodium hydroxide in the presence of Rochelle salt. The absorbencies were measured at 440 mμ on the spectrophotometer and the percentage of copper was determined from the calibration curve. The percentage of copper is plotted against the pH in Figure 1. In the range of pH 11.3 to 12.3, the measured copper content was within ±0.0025%.

Effect of Amount of Reagent. Theoretically, 1.5 ml. of a 0.5% α-benzoinoxime solution are necessary to combine with the copper in 0.5 gram of a 0.2% copper steel. Two milliliters were found to be sufficient, and an excess caused no error with plain steels. Absorption curves for the extracts from 2- and 10-ml. portions of the reagent showed no significant differences above 360 mμ. If the nickel content of the steel is over 0.25%, a large excess of reagent causes extraction of some nickel.

Effect of Amount of Rochelle Salt. The quantity of Rochelle salt should be held within the limits of 20 to 30 ml. of the solution specified. Incomplete extraction was obtained when the quantity was varied outside of these limits.

Effect of Standing. Three samples of steel containing 0.141% copper showed an average decrease of 0.002% copper after 30 minutes and a decrease of 0.003% copper after 75 minutes' standing.

Effect of Other Ions. Of the elements present in plain and low-alloy steels and cast irons, only nickel and cobalt were found to interfere. The absorption curves for 5 mg. (corresponding to 1%)

of nickel and cobalt with 2 and 10 ml. of reagent are shown in Figures 2 and 3.

Although 1% nickel would correspond to 0.02% copper when 2 ml. of reagent are used, the error is usually less, because of the equilibria existing with α-benzoinoxime, nickel, and copper. The following results were obtained on Bureau of Standards steels and irons:

Sample	% Ni	% Cu	% Cu Found	
			Beckman	Klett
34	0.30	0.122	0.126	...
107	0.81	0.074	0.066	0.069
32b	1.21	0.117	0.114	...
111	1.75	0.122	0.122	0.125
33a	3.24	0.080	0.068	0.072
126	36.42	0.096	0.026	...
Synthetic[a]	3.0	0.077	0.058	0.060
	2.5	0.071	0.062	0.063
	2.0	0.065	0.065	0.066
	1.5	0.059	0.056	0.059
	1.0	0.053	0.062	0.064

[a] Mixtures of Bureau of Standards steels No. 55 (0.041% Cu, 0.021% Ni) and No. 33a (0.080% Cu, 3.24% Ni).

These results indicate that the method is useful only for routine control analyses of ferrous alloys containing over 0.5% nickel.

In the case of cobalt, the interference is more serious.

Sample	% Co	% Cu	% Cu Found
Synthetic[a]	0.585	0.063	0.071
Synthetic[a]	0.28	0.062	0.064
153	8.45	0.099	0.036

[a] Mixtures of Bureau of Standards steels No. 65 (0.060% Cu) and No. 135 (0.099% Cu, 8.45% Co).

These results indicate that the method is useful for ferrous alloys containing up to 0.25% cobalt.

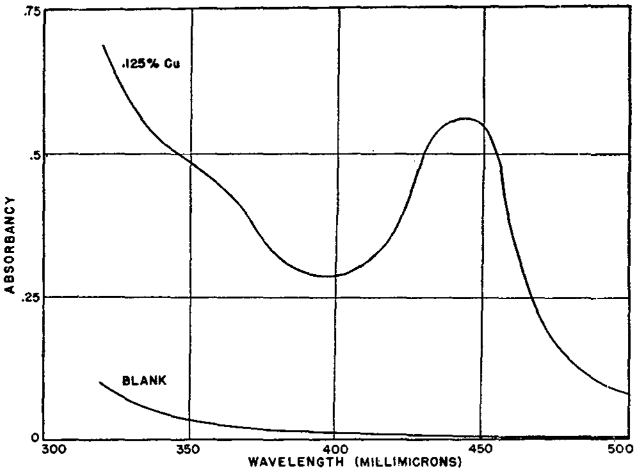

Figure 4. Absorbency of Copper-α-Benzoinoxime

SUMMARY

The method described is useful for the rapid determination of copper in many ferrous alloys. The chief interferences are nickel and cobalt, limiting the application of the method to plain and low-alloy steels and cast irons.

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Modified Molecular Weight Apparatus

ROBERT MATTESON
California Research Corporation, Richmond, Calif.

In laboratories handling a large volume of molecular weight determinations the rapidity with which the test may be performed is second in importance only to the accuracy. A solution to the problem in these laboratories has been effected through the redesign of the water-filled, differential vapor pressure thermometer of Menzies used in conjunction with a specially designed ebulliometer permitting a determination to be made with as little as 100 mg. of material. The operation is simple enough to be made by nontechnically trained personnel, and the time required for a test is of the order of 25 minutes. Using chloroform as a solvent, practically all of the high boiling types of materials encountered in petroleum research may be handled. The normal boiling point of the unknown must be at least 150° C. higher than the boiling point of the solvent. Changes in barometric pressure during the test do not affect the results. The equipment is standardized by four pure compounds covering the range from 128 to 890 in molecular weight. By a short extrapolation, molecular weights up to 1000 may be determined with an accuracy only slightly less than that obtainable using benzene in the cryoscopic method.

IN CONNECTION with many research problems, it is necessary to determine accurately the molecular weights of large numbers of samples. Often, particularly in industrial laboratories, speed is essential; costs must be held down and accuracy maintained at a high level. Older methods have not been able to fill all these requirements; hence, the Menzies-Wright (*4*) method was re-examined to ascertain if it could be adapted to the conditions mentioned above. As a result of this investigation, a modification of Menzies (*3*) differential thermometer has been successfully developed and a new ebulliometer designed for use with this thermometer.

Subsequent to the development of the apparatus described below, two excellent papers by Kitson and co-workers (*1*, *2*) appeared. They cover essentially the same ground as this paper, but their solutions of the problem are somewhat different mechanically. It is this difference that should prove of interest to workers in the field of molecular weights. For example, Kitson's apparatus is more complicated to fabricate, the heater is not easily replaced, and experience in these laboratories has shown that a traveling microscope rigidly attached to the frame holding the ebulliometer permits more accurate reading of the position of the meniscus of the liquid in the thermometer.

THERMOMETER

The Pyrex thermometer (Figure 1) is constructed by sealing the lower bulb to the drawn end of the 1.09-mm. inside diameter capillary. This bulb is approximately 30 mm. long and is made from 11-mm. outside diameter tubing. These dimensions are not critical, however. The total length of the capillary is 160 mm. In fabricating the thermometer a 6-mm. outside diameter tube is sealed to the upper end of the capillary. This tube is made a convenient length for handling.

Several liquids were tried in experimental thermometers before water was selected as the preferred filling material. Water is siphoned into the lower bulb, capillary, and upper bulb and then boiled out until the lower bulb is half full. At a point 25 mm. above the juncture of the capillary and the 6-mm. outside diameter tube the larger tube is sealed off to form the upper bulb. The 6-mm. tube is then cut off and a solid rod of equal diameter is sealed to the upper bulb. The male portion of the ground joint and the handle are sealed to the upper bulb as shown in Figure 1.

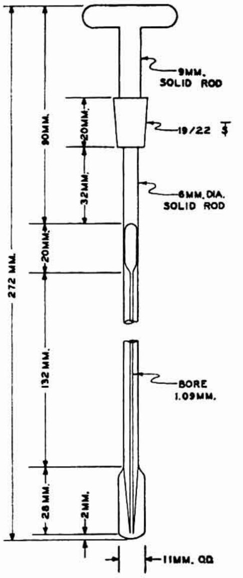

Figure 1. Differential Vapor Pressure Thermometer

With both bulbs of the thermometer at the same temperature, the upper meniscus of the liquid should stand about 50 mm. above the lower meniscus, the natural head due to capillarity. It is possible to adjust this height by cooling the lower bulb and bringing all the liquid into this bulb, then permitting the two bulbs to attain the same temperature, preferably in the ebulliometer containing the boiling solvent.

EBULLIOMETER

Figure 2 shows the ebulliometer designed specifically for use with the new thermometer.

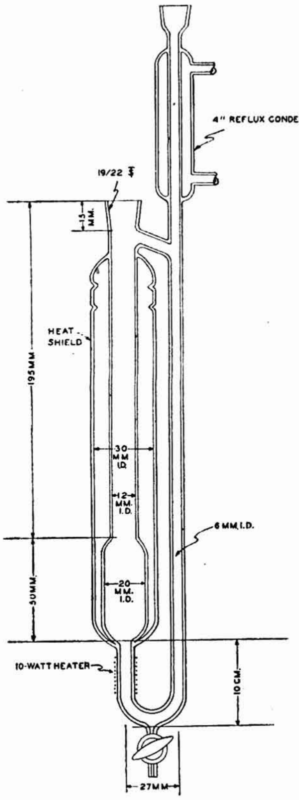

Figure 2. Ebulliometer

The 10-watt heater is made from 30 cm. (12 inches) of No. 26 Nichrome wire wrapped on the lower leg of the boiling section. Voltage across the heater is 10 volts supplied from a fixed-ratio transformer or a Variac. Where line voltage variations are troublesome it may be advisable to employ a voltage regulator, although such a step has not been necessary in this laboratory.

An unsilvered heat shield protects the boiler from drafts. The top of the boiler is fitted with a ground-glass joint into which is fitted the thermometer, thus affording constant positioning of the thermometer, a factor found to be important in attaining reproducibility of results.

A removable Drierite-filled drying tube is placed in the top of the condenser to prevent moisture from entering the ebulliometer during operation. The cock at the bottom of the return leg serves in draining the solution at the end of a test.

Before constructing the ebulliometer, there was some concern that possibly superheating of the liquid would result and make the design inoperable. Experience has proved that this is not the case, as evidenced by the negligible rise of the water column in the capillary of the thermometer when the pure solvent is boiling as compared with the height of the column when both bulbs are at room temperature.

COMBINED EQUIPMENT

Figure 3 is a photograph of the equipment in operation. A small 1-watt lamp operating at 10 volts is placed behind the boiler in order to illuminate the upper meniscus in the thermometer. A white translucent paper shield is placed between the lamp and the boiler to diffuse the light. Motion of the meniscus is observed by means of a Gaertner 32 × microscope mounted in a traveling micrometer slide capable of measuring to 0.01 mm.

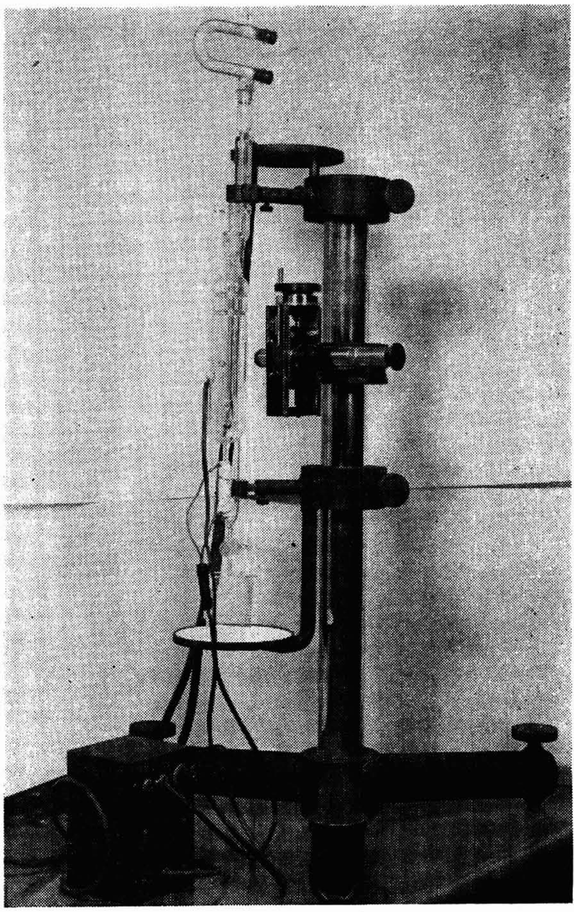

Figure 3. Apparatus in Operation

OPERATION

Solvent. In a petroleum research laboratory the preponderant type of sample for analysis is hydrocarbon or allied material. The preferred solvent is carefully dried chloroform of C.P. grade, which possesses the desired characteristics of relatively low boiling point and the power to dissolve an extremely wide range of substances. For over 4 years of operation in these laboratories, it has been found necessary to resort occasionally to only one other solvent, acetone. When acetone is used it is necessary to circulate ice water to the condenser; otherwise, tap water at room temperature (about 25° C.) suffices.

The present equipment uses 10 ml. of solvent in a determination of molecular weight.

Calibration. Each new batch of solvent is calibrated by determining the ΔS *vs.* mass curves for four compounds of known molecular weight. The preferred materials used in these laboratories are naphthalene (crystallized from alcohol), diphenyl bismuthine, tetrachlorobenzene, and tristearin. All chemicals are C.P. grade and cover the molecular weight range suitable for calibrating from $mw = 100$ to 1000.

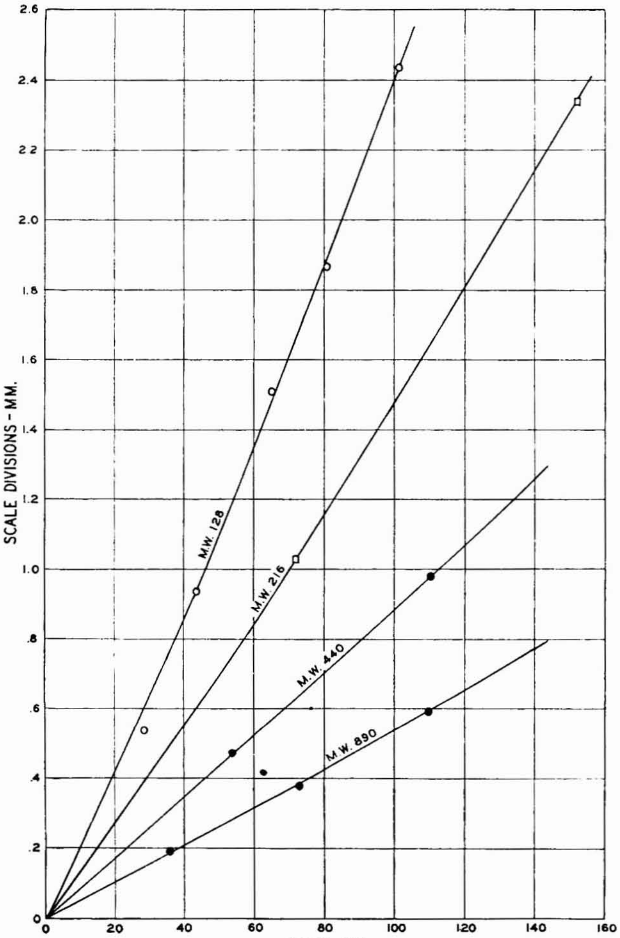

Figure 4. Calibration Curves for Differential Thermometer

In Figure 4 are plotted the ΔS *vs.* mass curves for a typical calibration. ΔS is the change in scale reading, or meniscus rise, for the addition of a given mass of solute to the solvent and is a measure of the increase in boiling point of the solution. In classical methods (*5*), $(\Delta T/m)$, where T is temperature and m is mass, is plotted against m to zero concentration, and the molecular weight is calculated from $\left(\frac{\Delta T}{m}\right)_{m\,=\,0}$ and the proper constant. However, experience with the new apparatus has proved it is better to plot, from the calibration curves, values of $\frac{\Delta S}{m}$ (taken at $m = 100$ mg.) against molecular weight, as shown in Figure 5, and to use this curve in determining molecular weights of unknowns. It has the advantage that the value for $\left(\frac{\Delta S}{m}\right)_{m\,=\,100}$ is obtained by interpolation, whereas $\left(\frac{\Delta S}{m}\right)_{m\,=\,0}$ is obtained by extrapolation, with the possibility of introducing much larger errors.

Determination of Molecular Weight. The first step in making a determination is to boil approximately 10 ml. of pure solvent in the ebulliometer for a reasonable length of time to clean it out. About 5 to 10 minutes have been found by experience to be sufficient time for this when working with clean stocks. Occasionally refractory materials require a longer cleaning time. The solvent from this cleaning run is drained through the cock at the lower end of the boiler and vacuum is applied at this point to hasten drying. Throughout the operation, the Drierite drier is in place as shown in Figure 3, and is removed only during introduction of solvent. With the ebulliometer clean, exactly 10 ± 0.01 ml. of solvent are charged and the heater is turned on, bringing the solvent to a boiling temperature.

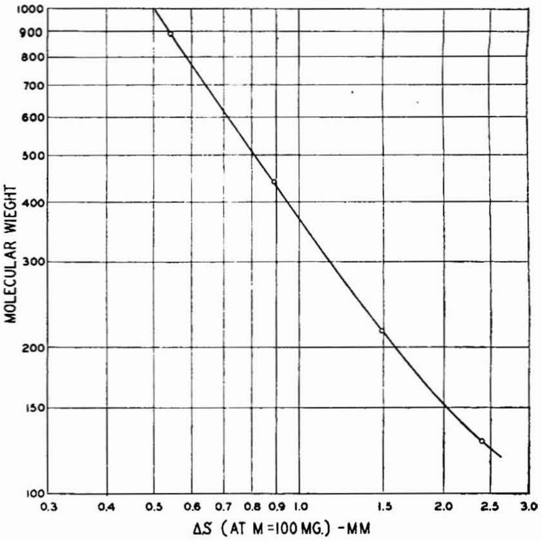

Figure 5. Standardization Curve for Differential Thermometer

Chloroform solvent

Table I. Comparison of Freezing Point Molecular Weight with Boiling Point Molecular Weight Determinations

Sample No.	Molecular Weight Freezing point ° C.	Molecular Weight Boiling point ° C.	Difference, %
1	192	195	+1.6
2	194	190	−2.1
3	218	218	0
4	237	245	+3.2
5	233	230	−1.3
6	251	250	−0.4
7	257	250	−2.7
8	264	270	+2.3
9	278	280	+0.7
10	416	425	+2.2
11	422	415	−1.7

Upon initial boiling the meniscus in the capillary of the thermometer rises immediately to the upper bulb, which is filled completely except for a tiny bubble that remains at the top. When the boiling vapors reach the upper bulb and the contents reach the temperature of the vapor, the upper meniscus is depressed into the capillary where it comes to rest at its equilibrium position. Boiling of the pure solvent is permitted to continue until the meniscus ceases moving, at which time the first portion of sample material may be introduced to the return leg of the ebulliometer.

In determining the molecular weight of a particular sample, approximately 50 to 60 mg. of the material are loaded into the end of a piece of 2-mm. inside diameter Pyrex tubing about 20 cm. in length, all material adhering to the outside of the tube is wiped off, and the tube and contents are weighed to 0.1 mg. A short piece of rubber tubing connected to the end of the glass tube opposite the sample facilitates handling and introduction of the sample into the ebulliometer. The tip of the glass tube holding the sample is lowered into the refluxing solvent which washes out the sample.

With the thermometer indicating that equilibrium has been obtained in the boiling solvent, the position of the meniscus in the capillary is noted by means of the traveling microscope and is recorded as S_1.

The drier is removed temporarily while this sample is introduced to the apparatus and is then replaced. The meniscus rises and comes to rest at a new position, which is noted and recorded as S_2. ΔS is then $S_2 - S_1$. A second 50 to 60-mg. portion of sample is next introduced into the ebulliometer and the third position of the meniscus is recorded as S_3. The value of ΔS

corresponding to the sum of the two masses is then $S_3 - S_1$. The ΔS *vs.* m curve is then plotted on rectangular coordinates. This method of plotting has the advantage that the origin gives an additional point through which the curve must pass and it becomes easier to detect flaws in the data.

From the curve thus constructed the value of $\left(\frac{\Delta S}{m}\right)_{m=100}$ is found and by reference to the standardized curve, such as Figure 5, the molecular weight is obtained.

A single determination of molecular weight can be made in a few minutes under ideal conditions. Where readily soluble compounds are being measured, and no cleaning difficulties are encountered, the average time required for a complete determination is about 25 minutes.

DISCUSSION

The change in the position of the upper meniscus is the only one followed in these measurements. This is done for purposes of simplification and is permissible, because the drop in the lower meniscus is a constant function of the rise in the upper meniscus. The method of plotting automatically takes care of the fact that ΔS is not $\Sigma \Delta S$. Actually, ΔS equals approximately 97% of $\Sigma \Delta S$ in practically all measurements made on this apparatus.

The usual precaution of maintaining an adequate difference between boiling point of solvent and solute must be observed, and in this laboratory no attempt is made to measure molecular weight by the ebullioscopic method of samples boiling below 210° C.—i.e., 150° C. above the boiling point of chloroform.

With careful attention to details, the accuracy attainable with this equipment is only slightly less than that obtained on the cryoscopic apparatus using benzene as a solvent and a Beckman thermometer for temperature measurement. A comparison of results obtained by the two methods on duplicate samples appears in Table I.

Certain types of compounds yield anomalous results, but this is common to the other widely used methods for determining molecular weight. In particular, the molecular weights of stearic acid and azoxybenzene are far too low when measured in the apparatus described here.

A word of caution is in order relative to the grease used in the ground joint of the thermometer. The silicone greases cause excessive foaming in the boiling solution and render the apparatus inoperable. A satisfactory grease is Celvacene Heavy, a product of Distillation Products, Inc., Rochester, N. Y.

Experience over the past 4 years has shown that this equipment meets all the requirements of speed, quality, and ruggedness in a laboratory where a large volume of molecular weight work is constantly being turned out.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the careful workmanship and ideas contributed by W. R. Doty in the construction of the glass equipment.

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Long-Chain Alkyl Sulfates

Colorimetric Determination of Dilute Solutions

FRED KARUSH[1] AND MARTIN SONENBERG[1]
New York University College of Medicine, New York, N. Y

A simple colorimetric method has been developed for the determination of long-chain alkyl sulfates. Solutions as dilute as 5×10^{-6} *M* can be analyzed with an accuracy of about 2%. The method depends on the formation of a complex between the detergent anion and the cationic dye rosaniline hydrochloride. This complex is extracted into a mixed organic solvent, 50% chloroform and 50% ethyl acetate, and its spectral absorption is read with a Klett-Summerson colorimeter. Pararosaniline hydrochloride is also suitable as a dye reagent. The molar sensitivity of the method increases from octyl to decyl to dodecyl sulfate, because of increasing efficiency of extraction of the dye-detergent complex with increase in chain length. The molar ratio of dye to detergent in the extracted complex is 1.

IN CONNECTION with a study of the interaction between bovine serum albumin and sodium octyl, decyl, and dodecyl sulfates ($C_nH_{2n+1}SO_4Na$), the need arose for an accurate analytical method for the determination of these alkyl sulfates in very dilute solutions, of the order of 10^{-5} *M*. A search of the literature revealed that no adequate method had been published.

The development of a colorimetric method was therefore undertaken, following the previous work of Brodie, Udenfriend, and Levy (*1*). As the basis of methods for the analyses of certain strong organic acids, these investigators utilized the formation of organic soluble complexes between the acids and the cationic dye rosaniline hydrochloride. The method described here is based on the formation by this dyestuff and pararosaniline hydrochloride of complexes with alkyl sulfates and their extraction into an organic phase. It has been applied to the determination of the alkyl sulfates noted above and is sufficiently sensitive to permit the analysis of solutions as dilute as 5×10^{-6} *M* (involving 2×10^{-8} mole) with an accuracy of about 2%. Its usefulness is not limited to alkyl sulfates and it can undoubtedly be applied to other strong organic acids which contain relatively large nonpolar groups.

MATERIALS

The rosaniline hydrochloride was obtained from the National Aniline Division, Allied Chemical and Dye Corporation, and was of 82% strength according to the manufacturer, the impurity being largely salt. A solution of the dye at a concentration of 4×10^{-4} *M* was prepared in 0.025 *M* phosphate buffer, pH 6.1. This solution was extracted several times with the same solvent

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[1] Present address, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

used in the analysis until the intensity of color in the organic phase remained unchanged on consecutive washings. The dye was then stored in a Pyrex bottle in the dark. The pararosaniline hydrochloride used in this study was a specially purified sample supplied by this manufacturer, and specified to be of 96.5% strength and to contain 2.5% moisture.

After some trials with a number of organic solvents, a mixed solvent consisting of 50% (by volume) chloroform (C.P.) and 50% ethyl acetate (C.P.) was selected for the extraction of the colored complex. The treatment of this solvent with aqueous alkali to remove organic acid was found to be an unnecessary precaution.

The spectral absorption of the complex was measured with the Klett-Summerson photoelectric colorimeter using a green (540) filter. However, it was found that the soft-glass absorption tubes ordinarily used with this instrument were undesirable because of the relatively high blanks they gave. Instead, specially made Pyrex tubes of outside diameter 14.0 ± 0.1 mm. were obtained and were found to give lower blanks than the Klett tubes when used in accordance with the procedure described below.

To minimize errors introduced by differences in the optical properties and dimensions of the Pyrex absorption tubes, zero and thickness corrections were established for each tube. Only tubes whose thickness correction was not more than 1% (of the Klett reading) were selected for use, thereby rendering this correction negligible.

For purposes of efficiency, simplicity, and the avoidance of contamination, it was desirable to carry out the extraction, centrifugation, and absorption measurement in the same tubes. This was possible with Pyrex tubes if they were treated before use with a boiling solution of approximately 1 *N* hydrochloric acid for about 1 hour. After this acid treatment the tubes were rinsed well with distilled water and dried in an oven for 10 to 15 minutes. Glass-stoppered Pyrex tubes were employed initially, but inverted serum caps (No. 2) were found to be as suitable as the glass stoppers. These caps were cleaned after use by soaking in 0.1 *N* hydrochloric acid solution, followed by thorough rinsing.

PROCEDURE

In a typical analysis eight or nine samples were simultaneously examined in triplicate with three blank controls. To each tube was added 1 ml. of the rosaniline hydrochloride solution (4×10^{-4} *M* in 0.025 *M* phosphate, pH 6.1). This was followed by the addition of the appropriate volume of alkyl sulfate solution in the same buffer, not exceeding 4 ml. The final volume of the aqueous phase was adjusted to 5 ml. and contained 0.025 *M* phosphate, pH 6.1. For the extraction 5 ml. of the mixed solvent were added and the tubes were stoppered and shaken by hand about 50 times.

Centrifugation for a few minutes resulted in the complete separation of the two phases with the organic phase, containing the colored complex, at the bottom. Its spectral absorption was then measured with the colorimeter against the reference tube filled with solvent. After the necessary corrections, including that for the blank, the amount of alkyl sulfate was read from the appropriate calibration curve.

Considerable variations in the conditions of the procedure can be tolerated. The use of pararosaniline hydrochloride does not lead to an increase of the blank nor does the use of 0.05 *M* phosphate buffer, pH 7.0. Furthermore, with dodecyl sulfate at least, the same sensitivity is observed with both dyes and under either set of buffer conditions.

Typical calibration curves for octyl, decyl, and dodecyl sulfates based on the procedure described above are shown in Figure 1. The upper scale refers to octyl sulfate and the lower one to the other sulfates. These curves were obtained with specially purified samples of alkyl sulfates supplied by the Fine Chemicals Division of E. I. du Pont de Nemours & Company. The calibration data are plotted in terms of the amount of alkyl sulfate used in the analysis.

DISCUSSION

Though the authors have not investigated in detail the nature of the dye-anion complex, it is fairly evident that its formation and extraction depend on the electrostatic interaction between opposite charges and the presence of a large nonpolar group in the organic anion. The significance of the latter is apparent from a comparison of the calibration curves in Figure 1. With increase in chain length the molar sensitivity of the method increases and the curves deviate less from linearity. This is, of course, precisely the behavior one would anticipate if the fraction of organic anion extracted into the organic phase as a colored complex increased with chain length. This conclusion has been verified experimentally by making a second extraction of the aqueous phase. The usual procedure was carried out with 4×10^{-8} mole of dodecyl sulfate and 10×10^{-8} mole of octyl sulfate to give net Klett readings of 309 and 326, respectively. A second extraction with 5 ml. of solvent was performed on 4-ml. aliquots of the aqueous phases including blank controls. The net Klett values obtained, 1 for the dodecyl compound and 175 for the octyl compound, clearly demonstrated the dependence of the efficiency of extraction on the chain length of the detergent.

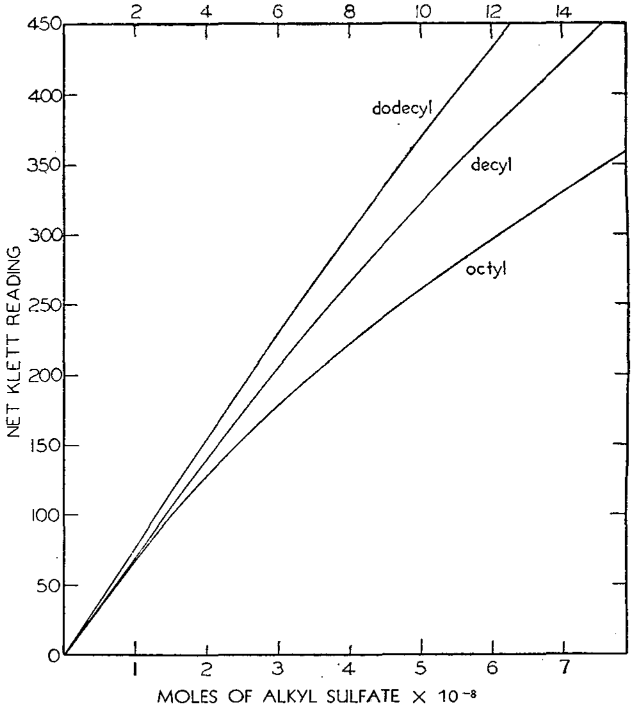

Figure 1. Calibration Curves

Inasmuch as only electrostatically neutral complexes would be expected to be extractable into the organic phase, it was inferred that the molecular ratio of dye to detergent in the complex is 1 to 1. This conclusion was subjected to experimental test by extracting 5 ml. of a buffered solution containing 4×10^{-8} mole of pararosaniline hydrochloride and 100×10^{-8} mole of dodecyl sulfate with 5 ml. of solvent. Because of the large excess of detergent, the dye was completely transferred to the organic phase and gave a net Klett reading of 326. On the other hand, with 4×10^{-8} mole of detergent a net reading of 307 was found by the usual procedure (involving dye excess). The ratio of the readings is 0.94, a value that is sufficiently close to 1 to establish the 1 to 1 stoichiometry of the dye-detergent interaction.

Whereas the drop in molar sensitivity is slight between dodecyl and decyl sulfates, the sensitivity for octyl sulfate is less than one half that for the other homologs. The concentrations of the alkyl sulfates used for analysis were far below the critical micelle concentrations (*2*, *3*).

One of the most persistent difficulties encountered in this study was the high and variable blanks. This was not due to the solubility of the dye in the organic solvent, but rather to contamination and absorption of the dye on the glass surface. By the use of Pyrex tubes and the procedure described above it was found possible to obtain satisfactorily low and consistent blanks. Over a period of weeks the average Klett readings for the blanks varied from 20 to 30, though in any particular series the individual readings generally agreed to within 2 units.

The dependence of the amount of colored complex extracted on the aqueous dye concentration was considerably different among the several homologs, but was consistent with the other differences noted above. A twofold increase in the dye concentration gave a 45% increase in the net Klett reading for octyl sulfate, about 8% for decyl sulfate, and practically none for dodecyl sulfate. Associated with this higher dye concentration was a slight increase in the blank reading. Increase of phosphate concentration from 0.025 *M* to 0.05 *M* yielded a 7% increase in the reading for octyl sulfate. No change in blank reading was observed. However, the presence of higher salt concentrations—e.g., 0.1 *M* sodium chloride—resulted in large increases in the blank.

The use of a mixed solvent instead of pure chloroform was dictated by the observation that the presence of ethyl acetate increased the efficiency of extraction of the colored complex. However, because its inclusion did give rise to higher blanks, a compromise figure of 50% was selected.

A few attempts were made to apply this method to long-chain fatty acids, but without success. Its failure in this case was undoubtedly associated with the weakly acidic character of the carboxyl group.

ACKNOWLEDGMENT

The authors are grateful to R. Keith Cannan for the generous hospitality afforded them in his laboratory during this investigation.

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RECEIVED August 8, 1949. Investigation conducted during tenure of a fellowship in cancer research of the American Cancer Society, recommended by the Committee on Growth, National Research Council.

Determination of Water in Glycols and Glycerol

CHARLES B. JORDAN AND VIRGIL O. HATCH
Paint and Chemical Laboratory, Aberdeen Proving Ground, Md.

A method has been developed for determining the percentage of water, by volume, in glycol-water or glycerol-water solutions. The procedure is a reflux distillation and salting-out process. 1-Butanol was selected as a refluxing medium. This solvent forms an azeotrope with water, but not with glycol or glycerol, and is capable of breaking the hydrates which are formed in aqueous glycol solutions. Forty samples of known composition, varying in water content from 1 to 95%, were analyzed. The method was found to be reliable and accurate. It is also applicable to mixed glycol-water solutions and mixtures of glycols with glycol-ethers and/or 1-butanol and alcohols containing more than four carbons. The method is especially applicable to permanent-type antifreeze solutions. It works equally well for new or used antifreeze solutions, with no interference from inhibitors or inorganic impurities.

IN PHASE rule studies of aqueous glycol or glycerol solutions, it is essential that the exact percentage of water be known. Boiling points, freezing points, and corrosive action on metals depend upon the amount of water in the solution. An industrial application of this type of problem exists in the investigation of permanent-type antifreeze solutions.

Several methods of determining water in different substances have been perfected, but no method could be found which would accurately and rapidly determine large percentages of water in glycol or glycerol samples. The accuracy of the Karl Fischer method of determining water (*3*) decreases rapidly as the water content exceeds 2%. The presence of dyes and certain inorganic salts affects the Karl Fischer method by giving false end points during titration. Therefore the method is not accurate for all antifreeze mixtures. Stevens and Nickels (*5*) determined small amounts of water in glycols and glycerol by means of a cloud point; however, this procedure is not applicable where large percentages of water are present.

A method of determining water in petroleum products (*1*), using the Dean and Stark apparatus (*2*) with toluene as the re-

Table I. Determination of Composition of Glycol, Glycerol, and Antifreeze Solutions

Run	Base	Temp.	Water Received	Known Water	Error	Butanol Received	Known Butanol	Error	Nonvolatile Constituent[a] Received	Nonvolatile Constituent[a] Known	Error
		° *F.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
1	Ethylene glycol	385	0.5	0.5	0	20.0	20.0	0	99.0	99.5	0.5
2	Ethylene glycol	385	30.0	30.3	0.3	20.0	20.0	0	69.5	69.7	0.2
3	Ethylene glycol	385	50.0	50.3	0.3	20.5	20.0	0.5	49.5	49.7	0.2
4	Ethylene glycol	385	70.5	70.2	0.3	19.5	20.0	0.5	29.5	29.8	0.3
5	Ethylene glycol	385	95.0	95.0	0	20.0	20.0	0	5.0	5.0	0
6	Antifreeze A	385	5.0	5.5	0.5	20.5	20.0	0.5	94.5	94.5	0
7	Antifreeze A	385	52.5	52.8	0.3	20.5	20.0	0.5	47.0	47.2	0.2
8	Antifreeze B	385	20.0	19.5	0.5	54.5	54.0	0.5	46.0	46.5	0.5
9	Propylene glycol	370	1.0	1.0	0	20.5	20.0	0.5	98.5	99.0	0.5
10	Propylene glycol	370	50.5	50.5	0	20.0	20.0	0	49.5	49.5	0
11	Diethylene glycol	450	2.0	2.0	0	19.5	20.0	0.5	98.5	98.0	0.5
12	Diethylene glycol	470	51.0	51.0	0	20.0	20.0	0	49.0	99.0	0
13	Antifreeze C	385	4.0	4.0	0	20.0	20.0	0	96.0	96.0	0
14	Antifreeze C	385	51.5	52.0	0.5	20.0	20.0	0	48.5	48.0	0.5
15	Glycol mixture	370	10.5	11.0	0.5	20.0	20.0	0	89.5	89.0	0.5
16	Glycerol	450	3.0	3.0	0	20.0	20.0	0	97.0	97.0	0
17	Glycerol	450	41.5	41.8	0.3	19.5	20.0	0.5	58.5	58.2	0.3
18	Glycerol	450	70.5	70.9	0.4	20.0	20.0	0	29.0	29.1	0.1

[a] Figure obtained by measuring volume of liquid remaining in distilling flask after distillation.

Table II. Determination of Water Content in Glycol, Glycerol, and Antifreeze Solutions

Run	Base	Temp.	Water Received	Known Water	Error
		° *F.*	*Ml.*	*Ml.*	*Ml.*
1	Ethylene glycol	350	0.5	0.5	0
2	Ethylene glycol	350	30.0	30.3	0.3
3	Ethylene glycol	350	50.0	50.3	0.3
4	Ethylene glycol	350	70.5	70.2	0.3
5	Ethylene glycol	350	94.5	95.0	0.5
7	Propylene glycol	350	1.0	1.0	0
8	Propylene glycol	350	50.5	50.5	0
9	Antifreeze D	350	31.5	32.0	0.5
10	Diethylene glycol	350	2.0	2.0	0
11	Diethylene glycol	350	51.0	51.0	0
12	Glycol mixture	350	10.5	11.0	0.5
13	Glycerol	350	3.0	3.0	0
14	Glycerol	350	41.5	41.8	0.3
15	Glycerol	350	70.5	70.9	0.4
16	2-Methyl-2,4-pentanediol	350	15.0	15.0	0

fluxing medium, could not be used, because toluene, water, and ethylene glycol form a ternary constant boiling mixture. Xylene was substituted for toluene but produced the same effect, as found by Trusler (*6*). The same difficulty was encountered when petroleum naphtha was substituted for toluene.

In their method of separating water and glycerol, Il'menev and Ovechkina (*4*) made use of the fact that 1-butanol formed a constant boiling mixture with water. For cases where the percentage of water was great, very large quantities of 1-butanol were required.

The reflux distillation method, as originally proposed by Dean and Stark (*2*), was adapted to the determination of water in the presence of glycols and/or glycerol by using 1-butanol as a refluxing medium. Tables I and II are representative of the results and accuracy that may be obtained by this method.

APPARATUS

The apparatus consists of a 250-ml. round-bottomed, short-necked flask, with standard-taper ground joint 24/40, having one side inlet tube for inserting a 580° F. thermometer. A Dean and Stark (Barrett type) receiver trap with stopcock and standard-taper ground joint 24/40, capacity 10 ml., is used. The water condenser is equipped with a male ground joint, standard-taper 24/40, and has a water-jacket length of 400 mm. A drying tube containing anhydrous calcium chloride is inserted into the top of the condenser. An electric heater of the rheostat type, 600-watt, or similar controllable heat source may be used (Figure 1).

PROCEDURE

Procedure I is a method for determining percentage, by volume, of water in a single known glycol or glycerol. Procedure II is a method for determining percentage, by volume, of water in an unknown glycol, a glycol mixture, or permanent-type antifreeze solution.

Procedure I. Transfer exactly 100 ml. of the sample to be tested into the round-bottomed flask, and add exactly 20 ml. of 1-butanol (distilling range 238° to 243° F.) and a few boiling chips. Insert the thermometer into the arm of the flask until the bottom of the bulb rests 0.25 inch (0.6 cm.) above the level of the liquid. Connect the flask to trap extension and insert the condenser. Set heat control so that the temperature will rise slowly but steadily until boiling commences, and adjust heat source to maintain a slow steady boil. For test samples having large percentages of water, it will be necessary to withdraw 5-ml. portions of water from the trap into a 100-ml. stoppered graduated cylinder at regular intervals. Care should be taken to maintain the lower layer (water layer) in the trap as high as possible during refluxing. Thus, the temperature of the flask cannot rise much above the boiling temperature of 1-butanol. Reflux for 2.5 hours and check the lower layer in the trap. Check again in 20 minutes. If no visible change in volume is noted, withdraw the water layer into the stoppered graduate.

Allow distillation to continue by increasing the heat source, if necessary, until the thermometer reaches a temperature 3° F. below the boiling point of the glycol. Butanol should be removed from the trap, into the same stoppered graduate containing the water, at regular intervals so as to prevent its flowing back into the flask. When the thermometer records a temperature 3° F. below the boiling point of the glycol, all the butanol will have been distilled; only glycol remains in the flask. Withdraw the remainder of the butanol into the graduate.

For samples containing large percentages of water, it will be necessary to use two 100-ml. glass-stoppered graduates. Any one graduate should not contain more than 80 ml. of liquid, leaving sufficient graduation to record volumes during the salting-out process.

CALCULATION. Record volume of liquid in the stoppered graduate to the nearest 0.5 ml. To this liquid, add 1 gram of anhydrous potassium carbonate. Stopper, shake well, and allow to settle. Read the volume of the butanol (top layer) to the nearest 0.5 ml. Add 1 gram more of anhydrous potassium carbonate, stopper, shake well, and allow to settle again. Again read the volume of butanol. If the butanol volume has increased add successive 1-gram portions of potassium carbonate until no increase in butanol content is noted. Record the volume of butanol, and subtract it from the original volume of liquid recorded. This will be the volume of water present in this sample and will be numerically equal to the percentage of water in the sample.

If during the reflux distillation, no water layer is formed and, upon the addition of 1 gram of potassium carbonate, no layer separates, allow the graduate to stand for 1 hour. If still no separation is noticed, this indicates that only a small amount, if any, of water is present in the sample. Pour the butanol into another graduate, allow for proper drainage, then read the new volume to the nearest 0.5 ml. Any water present will have been

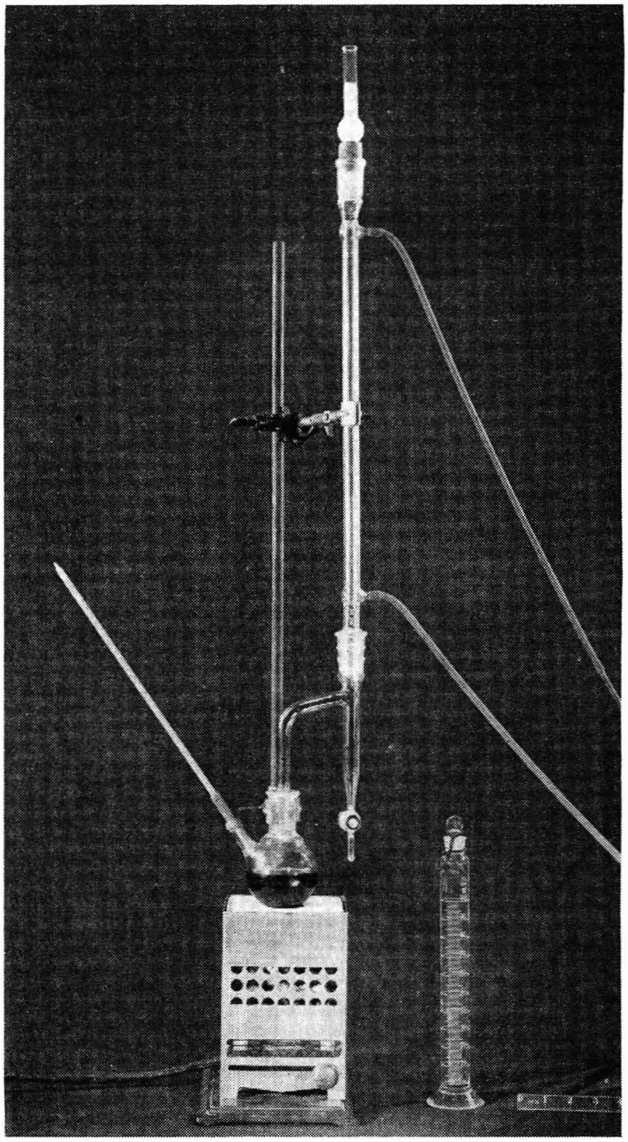

Figure 1

absorbed by the potassium carbonate. The difference in volumes of liquid in the two graduates will be the volume of water present in the sample and numerically equal to the percentage of water in the sample.

Subtract the calculated volume of water from the original 100-ml. sample. This will be the volume of glycol or glycerol in the sample and numerically equal to the percentage of glycol or glycerol in the sample.

The percentage of glycol or glycerol can be checked by properly draining the distilling flask, after cooling, and measuring the volume of liquid remaining in the flask (Table I).

Procedure II. For aqueous mixtures of glycols or antifreeze solutions of the permanent type containing polyhydric alcohols of unknown boiling point, it is possible to determine the water content by use of Procedure I with the following modification:

After the 2.5-hour reflux, or at the time when no increase in the water layer is noticed, remove the water layer from the trap. Continue the distillation until the temperature of the vapor just reaches 350° F., and withdraw all liquid in the trap. Perform salting out and calculations as before (Table II).

The presence of glycol ethers, 1-butanol or higher alcohols, or inorganic salts, does not interfere with this procedure.

ACCURACY

From Table I, based on glycols, glycerol, and antifreeze solutions of known composition, it can be seen that the maximum difference in volume received and known volume of water, butanol, or glycol is 0.5 ml. Table II, giving the water determination in compounds of presumably unknown composition, shows a maximum 0.5-ml. error.

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Determination of Sealing Strength of Paraffin Wax

C. S. FUNK AND H. D. DAVIS
Western Waxed Paper Company, San Leandro, Calif.
J. E. HANSON AND J. R. SEGESSER
California Research Corporation, Richmond, Calif.

In the manufacture of waxed heat-sealing wrappers—e.g., bread wrappers and carton wrappers—the sealing strength of paraffin wax plays an important part in establishing whether or not the finished paper will be satisfactory for its intended use. The method described offers a means of evaluating sealing strength with an accuracy within 5%. In most laboratories it is not possible to duplicate commercial waxing methods, but the test method presented here correlates with commercial results.

EVERY year a large quantity of waxed paper is used in wrappers for food products to protect foods against changes in moisture content during shipping and storage. Use has been made of the thermoplastic property of the wax coatings on these papers to provide heat-sealed closures on the packages. Typical examples are waxed bread wrappers and waxed carton overwraps. Automatic packaging machinery has come into common use for the application of these wrappers to the bread loaves or to the cartons. The heat seals are commonly made by passing the wrapped package first between heated plates and then between cooled plates, and applying pressure during both heating and cooling periods.

In order to be certain that a sufficiently strong heat-sealed closure will be provided by the waxed wrapper, it is necessary to be able to measure the sealing ability of the wax used, to compare waxes available from different sources, and to select a wax that can be counted on to meet the requirements of the particular wrapper.

Some indication of the heat-sealing ability of the wax can be obtained from measurement of its tensile strength. In general, in comparing paraffin waxes of similar melting point, it can safely be assumed that the wax with the highest tensile strength will possess the greatest sealing strength. Such an assumption is not considered correct, however, if waxes of different melting point or different composition are being compared.

A method of determining directly the heat-sealing ability (sealing strength) of the waxes obviously is desirable for making such comparisons.

One method for sealing strength determinations has been briefly described by E. I. du Pont de Nemours & Company (*1*). This method of sealing requires passing a roller over strips of waxed paper placed on a heated surface. The type of machine used to test the seal was not described. Padgett (*2*) recognized the importance of sealing strength and referred briefly to a test method. Other authors have mentioned sealing strength without presenting a complete method of evaluating it. This paper describes a laboratory method of applying uniform films of paraffin wax to paper, sealing the waxed sides of the paper together between hot glass plates, and measuring the force required to separate the heat-sealed papers.

APPARATUS

A supply of unwaxed opaque bread-wrap paper, preferably in roll form, 5 inches wide. The paper used in this series of tests was M.G. coated opaque sulfite bread-wrap weighing 25.5 pounds per ream (24 × 36 inches, 500 count).

Glassine paper, 24.0 pounds per ream (24 × 36 inches, 500 count).

Five steel disks, 7 inches in diameter, 1.25 inches thick, weighing 14 pounds, having one face flat and polished and the other fitted with a handle.

Five steel disks, 7 inches in diameter, 0.625 inch thick, and having one face flat and polished.

Disks of blotter paper 7 inches in diameter.

Oven, controlled to ±2° F. at 200° F.

Five glass plates, 0.25 × 4 × 4 inches.

Five glass plates, 0.25 × 5 × 5 inches.

Two glass trays, 2 × 6 × 10 inches for waxing paper.

Two racks for suspending waxed paper.

Paper cutter or sharp scissors.

Sealing strength tester (Figures 1 and 2).

American recording chart No. FO-150-8.

Planimeter for measuring the area within an irregular closed line.

CALIBRATION AND PROCEDURE

Adjusting Sealing Strength Tester. The following parts in Figures 1 and 2 are referred to when calibrating or adjusting the instrument:

1. Setscrew to adjust spring tension
2. Pen arm
3. Clip
4. Pulley
5. Adjustable lever arm
6. Setscrew
7. Setscrew

Normal use of the sealing strength tester requires the adjustment of setscrew 1 and pen arm 2. Setscrew 1 is adjusted until it just touches the spring, then pressure is increased by turning clockwise for three fourths of a turn. The pen arm is moved until it is about 0.25 inch from the zero circle of the recording chart, and final adjustment is made to the zero circle with setscrew 1. Static friction is overcome by gently tapping the instrument after each adjustment of the setscrew.

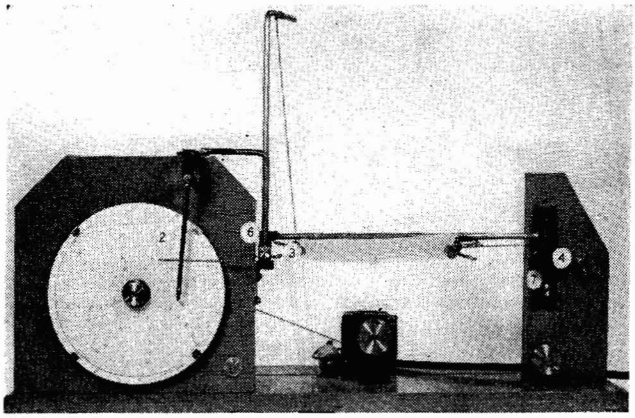

Figure 1. Sealing Strength Tester

Calibrating Sealing Strength Tester. The calibration is checked by suspending a 10-gram weight over pulley 4 by means of a small string or thread that is attached to clip 3. The instrument is tapped near the recording pen and near pulley 4 at the same time until a constant reading is obtained. The instrument was calibrated with a 10-gram weight for a deflection of 75 units on American recording chart No. FO-150-8. This value should be checked each day the instrument is used.

In order to calibrate the instrument a 10-gram weight is suspended over pulley 4 as described above. The deflection of the pen is adjusted to 75 by changing the length of lever arm 5. When the levels of setscrews on lever arm 5 are changed, clamp 3 (unloaded) is adjusted to the same height above the base by means of setscrews 6 and 7. These two setscrews allow the entire assembly between 6 and 7 to be moved vertically.

After a deflection of 75 units is obtained with a 10-gram weight, the instrument is calibrated with weights of 1 to 20 grams using the procedure required for checking the calibration. Figure 3 is a plot of the deflection radius against force in grams.

Procedure. Eight 18-inch strips cut successfully from a stub roll of bread-wrap paper are arranged in such a manner that the first, third, fifth, and seventh sheets are used with the wax to be tested. The alternate sheets are used with the standard wax. Alternate sheets are used in this manner to minimize the variations which normally might occur in the test paper. The test sample of wax is placed in a 6 × 10 inch tray and heated to 175° to 185° F. The paper is waxed on the glossy face only by passing over the surface of the molten wax. With practice the paper strips can be passed over the wax surface without running wax onto the back of the paper. The waxed paper strips are suspended from a rack to permit draining of wax across the paper while in the oven. The strips are then placed for 10 minutes in an oven set at 200° F. After oven treating, the strips of waxed paper are stacked with the glossy face up and cut into 5-inch sections. These sections are then cut to approximately 4 × 5 inch pieces with the 5-inch edge in the machine direction of the roll. The same procedure is used to prepare paper waxed with the standard wax.

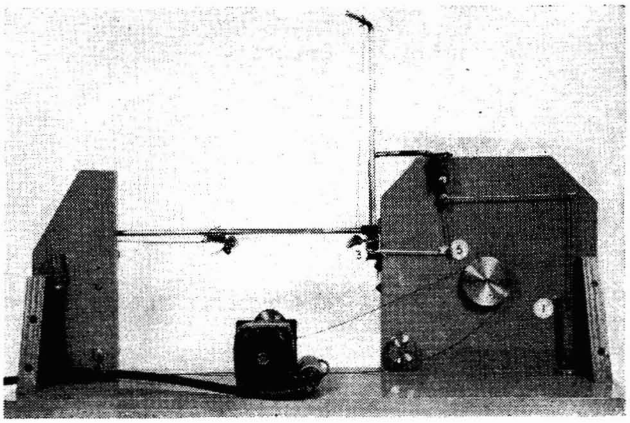

Figure 2. Sealing Strength Tester

Precautions. Wax should not be scraped off on the tray edge when the paper is waxed.

During oven treating care should be exercised to prevent the paper from touching the oven sides or sticking to itself, as this will result in erratic sealing strength values.

The tests should be completed within 1 hour after oven treating.

SEALING TEST

Two 4 × 5 inch sheets of waxed paper are placed with their glossy waxed sides together between two sheets of glassine paper. This waxed paper sandwich is placed between two glass plates preheated to 195° to 205° F. The bottom 5 × 5 inch glass plate rests on a 7-inch diameter steel disk and the top 4 × 4 inch glass plate is pressed down with a 14-pound, 7-inch diameter steel disk. The glass plates are insulated from the steel disks with blotter paper. After 5 minutes, the sealed sheets of waxed paper are removed and cut to exactly 3.5 × 4 inches, making sure that all edges are trimmed. The 4-inch dimension is in the machine direction of the paper.

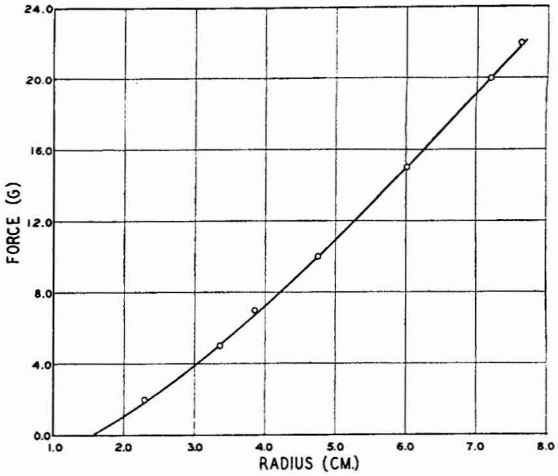

Figure 3. Calibration of Sealing Strength Tester

Before the laminated sheets are attached to the sealing strength tester, the pen arm is adjusted to zero on the recording chart. The sealed sheets are peeled apart about 0.5 inch across the 3.5-inch dimension and the resulting ends are attached to the clips on the horizontal bar. The motor is started and pulls the ends of the sealed sheets apart at the rate of 21 inches per minute. A record of the force required to separate the sheets is made on the recording chart. Strip width can be altered when necessary to eliminate extremely large or small deflections of the pen arm.

CALCULATIONS

The line inscribed by the recording pen is somewhat irregular in shape, as illustrated in Figure 4. To determine the average radius, a semicircular section is selected that excludes the first and last sections of the recorded curve. It is generally preferable to select a section that starts just after full load is applied.

An average radius of the sealing strength curve is calculated from the planimetered area of the semicircle. A mean radius is calculated for five determinations, discarding all value ±5% from this value and recalculating the mean. Five similar determinations are made using the standard wax.

Total force is obtained from the calibration curve and converted to grams per inch.

The sealing strength of the standard wax was established by averaging values obtained from 60 determinations. A correction is applied to the sealing strength of the test sample by means of the following equation:

$$S = \frac{A}{a} \times B$$

where S = corrected sealing strength of sample, A = standard sealing strength value of standard wax, a = measured sealing strength of standard wax, and B = measured sealing strength of test sample.

EXPERIMENTAL

Using the sealing test procedure described above, systematic changes were made at different important steps in the procedure to determine the variation in sealing strength. To eliminate changes due to the paper, a control run was made with paper from the same section of the roll. The refined paraffin wax used in all tests had a melting point of 147° F. (American melting point). A sealing strength of 2.6 grams per inch was established as the average of 60 determinations.

For all tests one factor was varied while the remaining conditions were the same as those used for the control.

It can be seen from Table I that an oven temperature of about 200° F. is required to drain the excess wax. At this temperature about 7 minutes are required for the wax in the paper to reach a constant level.

Table I. Oven Treatment at Various Temperatures and Times

Lab. Temp., ° F.	Oven Temp., ° F.	Oven Time, Min.	Wax on Paper, Lb./Ream[a]	Sealing Strength, Grams/Inch
75	160	5	17.2	3.7
75	160	10	13.1	3.4
76	180	5	13.1	3.1
75	180	10	..	2.9
72	200	2	13.1	3.0
83	200	5	11.6	2.7
82	200	7	10.1	2.6
77	200	10	10.3	2.6

[a] After oven treating.

Table II. Variable Waxing Temperatures

Lab. Temp., ° F.	Waxing Temp., ° F.	Wax on Paper, Lb./Ream	Sealing Strength
77	160	9.8	2.6
76	190	10.3	2.6
78	210	9.9	2.7

Table III. Effect of Aging Time

Aging Time, Hours	Aging Temp., ° F.	Sealing Strength
10 min.	77	2.6
2	77	2.7
24	77	2.8

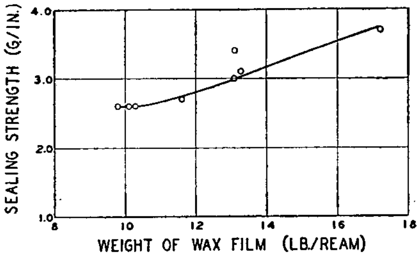

Figure 5. Effect of Wax Film Thickness on Sealing Strength

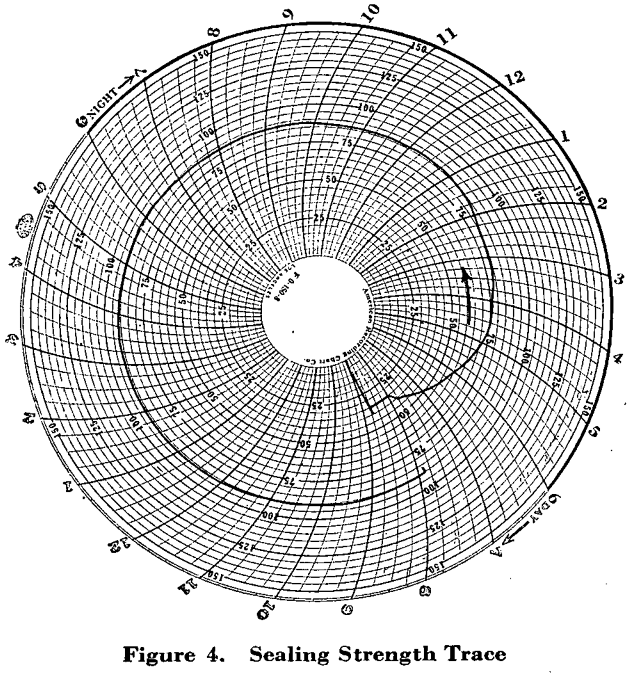

Figure 4. Sealing Strength Trace

Figure 5 illustrates the relationship between sealing strength and wax thickness on the test strips. The increase of sealing strength with an increase of wax thickness can be primarily attributed to the instrument characteristic of measuring paper stiffness as sealing strength. Near 10 pounds per ream of wax the sealing strength remains fairly constant at 2.6 grams per inch.

Sealing strength was not changed significantly when the bread-wrap paper was waxed at different temperatures, as shown in Table II.

The effect of aging time after oven treating and before sealing was determined at 10 minutes, 2 hours, and 24 hours. As shown in Table III, the sealing strength changes very slowly on aging.

Table IV. Conditioning Paper before Waxing

Relative Humidity, %	Storage Time, Hours	Sealing Strength, Grams/Inch
Anhydrous	24	2.6
45	24	2.6
81	24	2.6
100	24	3.4

In order to evaluate the effect of humidity on sealing strength, unwaxed bread-wrap paper was stored at constant humidities for 24 hours. Table IV indicates that sealing strength remained constant at 2.6 grams per inch up to 81% relative humidity. At 100% relative humidity the sealing strength increased to 3.4 grams per inch. This increase was probably due to the moist condition of the paper. Moderate changes in laboratory humidity from day to day should not materially alter sealing strength values.

The effect of sealing temperature on sealing strength is shown in Table V. The measured sealed strength increases with an increase in sealing temperature. The tests run at higher tempera-

Table V. Variable Sealing Temperature

Initial Temp., Glass Plates, ° F.	Sealing Strength, Grams/Inch
200	2.6
250	2.8
300	3.3

Table VI. Effect of Paper Variations

Stub Roll	Sealing Strength, Grams/Inch
1	3.1
2	2.9
3	3.1
4	2.5
5	2.5
6	2.8

tures indicated no advantage over those run at 200° F. Experimentally it is easier to work at the lower temperature.

Table VI demonstrates the change in sealing strength caused by variations in the paper. These variations can be corrected by the use of a control run with a standard wax. Each value was obtained using paper from a different stub roll. All the stub rolls were cut from one large roll.

DISCUSSION

In most laboratories, the preparation of waxed paper for a sealing strength test does not duplicate the conditions attained by machine-waxed paper for commercial use. With this in mind a test method should eliminate or reduce to a minimum the effect of as many variables as possible. The results of experiments conducted have shown that waxing temperature and moisture content of the paper before waxing for normal laboratory conditions do not affect sealing strength values. Hand waxing of paper produces an uneven film of wax, which results in erratic sealing strength values. If the paper is placed in an oven to allow the excess wax to drain off, reproducible results are obtained. It was found that 7 to 10 minutes at 200° F. resulted in a uniform wax film thickness. Oven treating also adjusts the humidity content of the paper to a common level, thus minimizing the effect of moisture content. Aging time after oven treatment results in a gradual increase but is within experimental error if the test is completed within 1 hour after oven treating. Although sealing strength increases with sealing temperature, the increase between 200° and 250° F. is only 8%. Therefore, sealing strength values are well within the limits of experimental error when small changes of plate temperature occur at 200° F.

No method of known accuracy was available for comparison with this method. The mean deviation from the mean of 60 tests was found to be less than 5% after correcting for deviation in results due to paper differences.

In addition to evaluating straight paraffins, this method has been used to compare the sealing ability of wax blends and to evaluate wax additives. Not only can tests be made on laboratory-waxed samples, but this same instrument has been useful in comparing machine-waxed samples. In this use, commercial machine-waxed samples are sealed together between hot glass plates in the manner described for oven-treated laboratory-waxed samples. The force required to separate the heat-sealed sheets is measured by means of the same sealing strength tester. An important use that has been made of this sealing strength tester is the investigation of customer complaints.

CONCLUSIONS

This test method permits the determination of sealing strength of different grades of paraffin waxes, and values can be correlated with results found in commercially waxed paper. With usage a minimum sealing strength can be established that will result in satisfactory seals for bread-wrap paper.

ACKNOWLEDGMENT

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Colorimetric Determination of Reducing Sugars with Triphenyltetrazolium Chloride

A. M. MATTSON[1] AND C. O. JENSEN

The Pennsylvania State College, State College, Pa.

TRIPHENYLTETRAZOLIUM chloride dissolves in water to form a colorless solution. Upon being reduced, this salt forms triphenylformazan, which is a red water-insoluble compound (*4*). Reducing sugars in an alkaline medium are capable of reducing triphenyltetrazolium chloride (*5*) and the quantity of formazan formed is proportional to the quantity of reducing sugars present. If time, temperature, and alkalinity are controlled, this reaction can be used as the basis of a convenient colorimetric method for the determination of reducing sugars.

REAGENTS

Sodium hydroxide solution, 1.00 *N*.

Acid-pyridine, 15 ml. of concentrated hydrochloric acid per 100 ml. of pyridine.

Tetrazolium reagent, 0.5% solution of 2,3,5-triphenyltetrazolium chloride in water. This reagent is somewhat sensitive to light. It is desirable to make it daily, although it may be kept for longer periods if stored in a dark cool place. (The tetrazolium salt can be secured from Arapahoe Chemicals, Inc., Boulder, Colo., or Panone Chemical Co., Farmington, Conn.)

APPARATUS

A constant temperature water bath maintained at 25° ± 0.1° C.

A photoelectric colorimeter with a 490 mμ filter or a spectrophotometer. The data reported in this article were secured with a Coleman Model 14 spectrophotometer using square cuvettes, 13 × 13 × 100 mm.

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[1] Present address, N. Y. Agricultural Experiment Station, Geneva, N. Y.

A convenient colorimetric method has been developed for the determination of sugars by the reduction of triphenyltetrazolium chloride and found applicable to the analysis of lactose in milk. The percentages of glucose and of fructose may be determined in a mixture of these sugars by using the Quisumbing and Thomas method and the proposed method. Glucose and fructose have been determined in honey by this method.

Satisfactory results were also secured when round cuvettes, 19 × 105 mm., were used.

SUGAR SOLUTIONS

The procedure employed will give readings between 10 and 85% transmittance with the following concentrations of sugars (mg. per 10 ml.): lactose 3 to 30, fructose 0.3 to 2.7, glucose 1.5 to 19, invert sugar 0.4 to 4.7. The purity of each sugar used was assumed to be the purity found as the result of an analysis by the Quisumbing and Thomas method. Lactose concentrations are expressed as anhydrous lactose.

PROCEDURE

The sugar solution to be analyzed (10 ml.) is introduced into a dry 50-ml. Florence flask and placed in a water bath, maintained at 25.0° C., for 10 minutes to allow the flask and solution to reach a constant temperature. A blank solution using distilled water is also placed in the water bath.

Ten milliliters of alkali are added to each flask and the time is recorded. The flasks are swirled to mix the solutions, returned quickly to the water bath, and held in the bath for 6 minutes to allow equilibrium temperature to be reached.

At the end of 6 minutes, 2 ml. of the tetrazolium salt solution are added to each flask. The flasks containing the solutions are agitated and returned to the water bath for exactly 30 minutes after the addition of the tetrazolium salt.

At the end of this period 10 ml. of acid-pyridine are added to each flask. The pyridine dissolves the formazan and a clear red solution results, except in the case of the blank which is colorless.

The per cent transmittance is read at a wave length of 490 $m\mu$ by means of a photoelectric colorimeter or a spectrophotometer. From this value the sugar content can be determined by means of a standard curve previously prepared.

FACTORS INFLUENCING THE DETERMINATION

Alkalinity. The alkalinity of the reaction is one of the less critical factors. The 1 *N* sodium hydroxide used may vary by ±3% without causing an appreciable error.

Temperature of Reaction. Because triphenyltetrazolium chloride is readily reduced by reducing sugars at room temperature in alkaline solutions, 25° C. was chosen as the standard reaction temperature. The reaction is sensitive to rather small changes in temperature. A change of ±1° C. from 25° C., for instance, will result in an error of about 10% in a lactose determination. By controlling the water bath to ±0.1° C. the error in per cent transmittance due to temperature variations is reduced to 1% or less.

Length of Reaction Time. The reaction time was chosen for convenience as 30 minutes. This reaction time did not allow too much color to develop and furthermore allowed a number of samples to be run simultaneously. In order to control the variation in transmittance to less than 1%, it is necessary to limit differences in the length of reaction time to ±30 seconds.

The reaction is terminated at a definite time by adding acid to lower the alkalinity of the medium below that at which the reaction will occur at room temperature. By incorporating the acid and the pyridine in one reagent, the reaction is stopped and the formazan is dissolved in one step. The color developed under these conditions is stable for several hours if the solution is kept out of direct skylight.

Wave Length. The preparation of a spectral-transmittance curve showed that the red solution resulting from the reduction of the tetrazolium reagent by glucose followed by the addition of pyridine had a minimum transmittance at 480 to 500 $m\mu$. A similar study on a solution of recrystallized triphenylformazan dissolved in chloroform gave a value of 490 $m\mu$ as the wave length of minimum transmittance. Therefore 490 $m\mu$ was chosen as the wave length for transmittance measurements.

STANDARD CURVES

Standard curves for lactose, invert sugar, fructose, and glucose were established. Upon plotting the optical density $(2 - \log G)$ against sugar concentration, straight-line graphs passing through the origin were obtained. The slopes of these lines were evaluated by averaging the individual K values for each particular sugar concentration used. The K values were calculated by the equation:

$$K = L/C \qquad (1)$$

where L equals optical density $(2 - \log G)$, and C equals sugar concentration in milligrams per 10 ml. of sample solution.

The K values for lactose, glucose, fructose, and invert sugar are presented in Table I. A comparison of the amount of lactose found by using the determined K value with the amount actually present in known lactose solutions is shown in Table II.

Table I. Relative Reducing Power of Some Reducing Sugars

		Relative Reducing Power	
	K	Tetrazolium method	Quisumbing and Thomas method
Lactose	0.0268	0.523	..
Glucose	0.0512	1.00	1.00
Fructose	0.362	7.07	0.92[a]
Invert sugar	0.212	4.14	..

[a] In range 100 to 150 mg.

Table II. Determination of Lactose

Sample	Present, Mg.	Found, Mg.	Sample	Present, Mg.	Found, Mg.
1	24.7	24.9	9	13.8	14.1
2	23.7	23.4	10	12.4	12.4
3	22.3	22.3	11	10.4	10.3
4	20.7	21.2	12	9.9	9.7
5	19.8	19.8	13	7.4	7.4
6	18.5	18.7	14	6.9	6.7
7	17.8	18.0	15	6.2	6.1
8	14.8	14.9	16	5.9	5.8

ANALYSIS OF MILK

Milk samples were clarified by the addition of neutral lead acetate (*2*) followed by ammonium oxalate. The copper sulfate method of clarification could not be used, as it interfered with the subsequent determination of lactose by the proposed method. The clarified filtrate was used for analysis by both the proposed method and the Quisumbing and Thomas method (*1*). The 10-ml. aliquot used for the proposed method corresponded to 0.5 gram of milk. The per cent lactose was calculated by use of Equation 1, substituting the value 0.0268 for the constant K. A comparison of the results obtained with milk samples by using the proposed method and the Quisumbing and Thomas method is shown in Table III.

Table III. Lactose in Milk

Sample No.	Lactose, % Tetrazolium method I	Lactose, % Quisumbing and Thomas method II	Ratio I/II
1	4.44	4.34	1.023
	4.44		1.023
2	4.25	4.32	0.984
	4.31		0.998
3	4.39	4.39	1.000
	4.36		0.993
4	4.28	4.29	0.998
	4.31		1.005
5	4.56	4.54	1.006
	4.56		1.006
6	4.53	4.48	1.011
	4.50		1.006
7	4.76	4.65	1.024
	4.76		1.024
8	4.76	4.69	1.017
	4.76		1.017
9	4.19	4.07	1.030
	4.16		1.022
10	4.16	4.09	1.017
	4.13		1.010
11	4.25	4.17	1.019
	4.25		1.019
12	4.37	4.28	1.021
	4.31		1.007
13	4.46	4.38	1.018
	4.43		1.011
14	4.43	4.35	1.018
	4.37		1.005
15	4.40	4.32	1.018
	4.40		1.018
16	4.46	4.35	1.025
	4.40		1.011
		Total	32.404
		Mean	1.0126

DETERMINATION OF GLUCOSE AND FRUCTOSE IN MIXTURES

The relative reducing power of fructose to glucose (Table I) varies greatly between the proposed method and the Quisumbing and Thomas method. Using the proposed method, fructose has about seven times the reducing power of glucose, whereas when using the Quisumbing and Thomas method fructose is only 92% as active as glucose in the range of 100 to 150 mg. of sugar. The relative activity of glucose and fructose in a mixture of these sugars has been found to differ somewhat from the relative reducing power of the single sugars. In mixtures with glucose-fructose ratios of 20–80 to 60–40 the relative reducing power of fructose was found to be 7.40 times that of glucose. Using values obtained from sugar determinations by both the Quisumbing and Thomas and the tetrazolium methods it is possible to calculate the percentages of glucose and fructose in a mixture of these sugars.

The development of the equations necessary for the calculation of glucose and fructose in a mixture is shown below.

$$S = aF + G \tag{2}$$

$$S' = a'F + G \tag{3}$$

where

S and S' are equal to the apparent per cent total sugars expressed as glucose by the tetrazolium method and the Quisumbing and Thomas method, respectively.

a and a' are constants denoting the relative reducing power of fructose in a mixture of fructose and glucose by the respective methods.

F equals per cent of fructose.

G equals per cent of glucose.

Subtracting Equation 2 from 3:

$$(S - S') = (a - a')F \tag{4}$$

$$(S - S') = AF \tag{5}$$

where A is a constant that can be evaluated by substituting experimentally determined values of S and S' corresponding to known sugar concentrations.

The value of A so determined was found to be 6.48.

After the value of constant A has been established, Equation 5 can be used to calculate the percentage of fructose in unknown mixtures of glucose and fructose from experimental values of S and S'. The per cent glucose may be determined by substituting the value 0.92 for constant a' in Equation 3 and solving this equation for G after the per cent fructose has been determined. Experimental values obtained using known sugar mixtures are shown in Table IV.

ANALYSIS OF HONEY

The percentages of glucose and fructose in honey were determined by using the method previously outlined for determining glucose and fructose in a mixture of these sugars (Equations 3 and 5).

The honey was clarified with dialyzed cream of alumina according to the usual procedure (*3*). A rapid method of inversion by heating after the addition of acid was used. Ten-milliliter samples equivalent to 3 mg. of honey were used for the proposed

Table IV. Mixtures of Glucose and Fructose

(Basis, 100% total reducing sugars)

Actual Glucose, %	Experimental Values, % Glucose	Experimental Values, % Fructose	Experimental Values, % Total sugars
20	20.8	78.9	99.7
40	39.2	61.0	100.2
50	50.9	49.3	100.2
60	59.5	40.4	99.9

Table V. Determination of Glucose and Fructose in Honey

Sample No.	Apparent Total Sugars as % Glucose: Tetrazolium method, $S(ap)$	Apparent Total Sugars as % Glucose: Quisumbing and Thomas method, $S'(ap)$	Fructose, %	Glucose, %	Total Sugars: Combined methods, %	Total Sugars: Quisumbing and Thomas method, % (invert sugar)
1	320	68.8	38.7	33.2	71.9	72.3
	318		38.4	33.5	71.9	
2	314	66.8	38.1	31.8	69.9	70.2
	307		37.1	32.7	69.8	
3	297	63.9	36.0	30.8	66.8	67.2
	297		36.0	30.8	66.8	
4	311	67.0	37.7	32.4	70.1	70.4
	314		38.1	32.0	70.1	
	316		38.4	31.7	70.1	
	314		38.1	32.0	70.1	
5	311	66.5	37.7	31.9	69.5	69.9
	314		38.1	31.5	69.6	
	311		37.7	31.9	69.5	
	309		37.4	32.1	69.5	
6	322	69.2	39.1	33.2	72.3	72.7
	320		38.8	33.5	72.3	
	324		39.4	33.0	72.4	
	318		38.4	33.9	72.3	
7	320	69.7	38.6	34.2	72.8	73.1
	318		38.3	34.5	72.8	
	318		38.3	34.5	72.8	
	320		38.6	34.2	72.8	

method, and a sample containing 150 mg. of honey was used for the Quisumbing and Thomas method. The results are found in Table V.

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Determination of Certain Nitrogen-Containing Functional Groups in Organic Compounds

WALTER W. BECKER
Hercules Experiment Station, Hercules Powder Company, Wilmington 99, Del.

Nitrogen exists in a considerable number of combinations with oxygen, hydrogen, and carbon in organic compounds. It is often necessary to determine the specific nitrogen-containing functional group present. This paper presents a review of available methods for determining certain of these groups. Included are literature references and an outline of the procedures for the determination of the nitrate, nitro, diazo, azo, amino, amide, alkimide, and nitrile groups in organic compounds.

NITROGEN exists in organic compounds in probably more combinations with oxygen, hydrogen, carbon, and itself than any other element. Its several valences enable it to unite with these other elements to form a variety of nitrogen-containing functional groups. In early days only the total nitrogen could be determined by the Kjeldahl or Dumas methods; as time went on, methods for determining the specific groups began to be developed.

This article presents a review of the available methods for the specific determination of certain of these groups, which have been arbitrarily divided into a number of classes for the purpose of presentation. The principle of the procedure, a few typical compounds which have been analyzed, and literature references are given.

NITRATE NITROGEN (—ONO_2)

The methods for determining nitrate nitrogen fall into three general classifications.

By Nitrometer. The apparatus in general use today is the Du Pont compensating-type nitrometer (*29*), which is a modification of Lunge's gasvolumeter (*21*). With the du Pont nitrometer, the nitric oxide generated may be readily brought to the volume it would occupy at 20° C. and 760 mm.; this eliminates the need for temperature and barometric corrections. Elving and McElroy (*10*) developed a semimicronitrometer of the compensating type, with a motor-driven shaker. The nitrometer is usually standardized with potassium nitrate, which reacts with sulfuric acid and mercury according to the following equation:

$$2KNO_3 + 4H_2SO_4 + 3Hg \longrightarrow K_2SO_4 + 3HgSO_4 + 4H_2O + 2NO$$

In assembling the nitrometer, care must be taken to dry all glassware and to use clean mercury. As a safety precaution, a cellulose acetate face mask must be worn during the generation of the nitric oxide, in case of an explosion. When carefully trained, the average analyst is able to operate the nitrometer safely. Complete directions for assembling and standardizing the Du Pont nitrometer, as well as the procedure for analyzing samples, are given by Scott (*34*) and by the American Society for Testing Materials (*3*).

The method can be applied to the analysis of nitroglycerin, nitrocellulose, pentaerythritol tetranitrate, and similar esters, or to compounds that liberate nitric oxide quantitatively. Obviously, the method is inapplicable to nitrate nitrogen-containing compounds that liberate carbon dioxide, sulfur dioxide, or gases other than nitric oxide. Nitrogen in finished explosives usually cannot be determined by means of the nitrometer because they invariably contain stabilizers such as diphenylamine or diethyldiphenylurea. These stabilizers undergo partial nitration in the decomposing bulb, and cause low results. However, certain compounds undergo quantitative nitration under these conditions. For example, if weighed amounts of potassium nitrate and material containing mononitrotoluene are shaken with sulfuric acid in the decomposing bulb, quantitative nitration to dinitrotoluene takes place. The decrease in volume of the nitric oxide obtained is a measure of the mononitrotoluene present.

Saponification Methods. The reduction of inorganic nitrates to ammonia by the use of Devarda's alloy is well known (*2*). It would seem, then, that organic esters such as nitroglycerin and nitrocellulose could be saponified with sodium hydroxide, and then Devarda's method applied. Unfortunately, when such esters are treated with sodium hydroxide, various reduction products including ammonia are formed.

Muraour (*24*) avoided this difficulty by conducting the saponification in an oxidizing medium. He dissolved the sample in acetone, added sodium hydroxide, sodium perborate, and hydrogen peroxide, then let stand overnight for the saponification to sodium nitrate. He then added Devarda's alloy, heated, and distilled the resulting ammonia into standard acid (*2*).

Considerable foaming occurs at the start of the distillation, due to the presence of a floating layer of isophorone which is formed by reaction of acetone and sodium hydroxide. A good distilling head must, therefore, be used to prevent entrainment of alkali during distillation.

Muraour's procedure has been used in this laboratory for various products, with good results. It is particularly applicable to samples of lacquer containing nitrocellulose; in this case the nitrogen content of the nitrocellulose must be known or assumed in order to calculate the amount of the latter present.

Volumetric Reduction Methods. Knecht (*16*) first determined nitric acid in aqueous solution by reduction to nitric oxide with ferrous iron in hydrochloric acid solution, then titrating the resulting ferric iron with a standard solution of titanous chloride, using ammonium thiocyanate indicator. In this laboratory, it was found that Knecht's method could be used for the determination of the nitrate nitrogen in nitroglycerin and nitroglycol, after dissolving the sample in glacial acetic acid (*4*). The procedure could be applied in the presence of aromatic nitro compounds such as 2,4-dinitrotoluene; the stabilizers diphenylamine and diethyldiphenylurea did not interfere.

The equations in the case of nitroglycerin are as follows:

$$C_3H_5(ONO_2)_3 + 9FeCl_2 + 9HCl \longrightarrow C_3H_5(OH)_3 + 9FeCl_3 + 3NO + 3H_2O$$

$$TiCl_3 + FeCl_3 \longrightarrow TiCl_4 + FeCl_2$$

NITRO NITROGEN ($-NO_2$)

The methods for determining the nitro group depend upon the three general types of organic nitro compounds; more than one method is applicable to each type.

Nitro Aromatic Compounds. The reduction of the nitro group by means of titanous chloride solution was first developed by Knecht (*16*) and applied to various compounds by English (*11*) and Callan and Henderson (*6*). The compound is dissolved in water or alcohol and an excess of standard titanous chloride is added. After boiling, the excess is titrated with standard ferric iron solution, using ammonium thiocyanate indicator, thereby obtaining a measure of the nitro nitrogen present. Six equivalents of titanous chloride are required for each nitro group.

Certain nitro compounds do not reduce in the orthodox manner with titanous chloride. For example, in hydrochloric acid solution, low results for α-nitronaphthalene are due to the formation of 1,4-monochloronaphthylamine. Nitroanisole becomes chlorinated in a similar manner. Using titanous chloride and sulfuric acid, English (*11*) obtained low results for nitro hydrocarbons such as the mononitro derivatives of benzene, toluene, and xylene. Callan and Henderson (*6*) showed that accurate results could be obtained on the foregoing types of mononitro hydrocarbons by using titanous sulfate in place of the chloride, and a reflux condenser to avoid loss of the sample by steam distillation.

For compounds such as dinitrotoluene and trinitrotoluene, a 5-minute boiling period is necessary in acid solution. With the use of an alkaline buffer such as sodium citrate, Kolthoff and Furman (*17*) state that the reduction will take place practically instantaneously at room temperature. This shortened and equally accurate procedure was used at ordnance plants operated by the Hercules Powder Company (*5*) during World War II.

The polarograph may also be used to determine the nitro group; the reduction at the dropping mercury electrode probably proceeds as follows in the case of nitrobenzene:

$$C_6H_5NO_2 + 6H^+ + 6e \longrightarrow C_6H_5NH_2 + 2H_2O$$

Shikata (*36*) and his associates measured the reduction potentials of mono- and dinitrobenzene, mono- and dinitrophenol, and the nitranilines, with satisfactory results. Kolthoff and Lingane (*18*) summarize the application of the polarograph to nitro compounds; the E. H. Sargent Company (*33*) publishes frequently a review of the literature concerning the applications of the polarograph.

Nitro Aliphatic Compounds. The nitro group in this type of compounds is much more difficult to reduce. The total nitrogen content may be determined by the Dumas method or by suitable modifications of the Kjeldahl method. A study of the available references listed in the review article by Hass and Riley (*14*) did not reveal any reliable volumetric reduction methods for the specific determination of the nitro group in nitroparaffins.

DeVries and Ivett (*9*) developed a polarographic method for the quantitative determination of several low-molecular-weight nitroparaffins, when present in dilute solution. A 0.05 *M* sulfuric acid solution was used as the supporting electrolyte. The half-wave potentials *vs.* a normal calomel electrode ranged from 0.60 to 0.72 volt, for the series nitromethane to nitrobutane. The half-wave potentials were not sufficiently far apart to determine the compounds separately in the presence of each other. Heyrovský and Novak (*15*) used the polarograph in connection with an investigation of the stability of smokeless powder, to determine oxides of nitrogen and nitric acid. Nitroglycerin interfered but could be removed by solvent extraction.

Nitroamino Compounds. In connection with the analysis of newer explosives compositions during World War II, this laboratory worked on the development of methods for the determination of nitroguanidine and cyclotrimethylenetrinitramine, popularly known as RDX. In the analysis of nitroguanidine, the regular titanous chloride reduction method gave erratic results. On increasing the acidity and lengthening the boiling time to 15 minutes, it was found that 1 mole of nitroguanidine reacted with 4 moles of titanous chloride (*19*).

Cottrell, MacInnes, and Patterson (*8*) determined the nitrogen content of nitroguanidine by dissolving the sample in concentrated sulfuric acid and titrating with ferrous sulfate solution. The visual end point was not sufficiently reproducible, but by using a platinum-tungsten electrode system, they were able to obtain concordant results by titrating potentiometrically. Apparently, when dissolved in sulfuric acid, the nitroguanidine yields nitrate ion under the given conditions.

Cope and Barab (*7*) were able to analyze nitroguanidine and tetryl by the nitrometer method; Elving and McElroy (*10*) also analyzed these two compounds. Cope and Barab postulated that the nitro or nitroso group must be attached to a second nitrogen atom, which in turn is attached to a carbon atom. For example, they were able to determine the nitroso group on diphenylnitrosoamine, which fulfills these conditions, but found that no reaction occurs in the nitrometer with *p*-nitrosodimethylaniline or nitromethane.

Rathsburg (*31*) used titanous chloride to analyze "the nitration product of hexamethylenetetramine" (cyclotrimethylenetrinitramine or RDX) and formulated the reaction as follows:

$$C_3H_6N_3(NO_2)_3 + 12TiCl_3 \longrightarrow C_3H_6N_6$$

He did not balance the equation.

In this laboratory, titanous chloride alone gave low results on RDX. Ferrous chloride was tried, but the reduction was negligible. However, by the addition of both reducing agents and a 30-minute boiling period, reduction was complete, 4 equivalents of titanous chloride being required for each nitro group (*19*). In view of these results, it seems likely that Rathburg used titanous chloride which contained ferrous iron as an impurity.

DIAZO AND AZO NITROGEN ($N{\equiv}N{=}$ AND $-N{=}N-$)

Diazo. Knecht and Hibbert (*16*) successfully titrated benzenediazonium chloride with titanous chloride and found that 4 equivalents were required. Wienhaus and Ziehl (*43*) treated the ethyl ester of diazoacetic acid with hydrogen in the presence of palladium catalyst, and measured the nitrogen liberated. Pierce and Rising (*28*) modified Mehner's (*22*) method and adapted it to a micro scale. The sample is heated with hydrochloric acid in a closed system, and the gaseous nitrogen formed is measured in an azotometer. On diazoaminobenzene and three substituted diazoaminobenzenes, they obtained values which agreed closely with the total nitrogen present as determined by another method. The method should be applicable to any substance that evolves nitrogen quantitatively under these conditions.

In this laboratory, methods for the determination of diazo and nitro nitrogen in diazodinitrophenol were devised (*35*). Rathsburg (*31*) had formulated the reduction of this compound by titanous chloride as follows:

$$C_6H_2O(NO_2)_2N_2 + 13TiCl_3 \longrightarrow C_6H_2O(NH_2)_2NNH_2$$

The author's results confirmed the stoichiometry of Rathsburg's equation, but no differentiation between the diazo and nitro nitrogen could be calculated. On treating diazodinitrophenol with hydrochloric acid or sodium hydrosulfite and measuring the evolved nitrogen gasometrically, low results were obtained. However, when a large excess of titanous chloride was added to the sample, the mixture heated, and the evolved nitrogen measured, quantitative results were obtained on diazodinitrophenol. Rathsburg's unbalanced equation is apparently in error.

Azo Nitrogen (—N═N—). Knecht (*16*) was able to analyze a variety of "azo dyestuffs" with titanous chloride, either by titrating directly, or by adding an excess of the reducing agent, boiling, and back-titrating with ferric solution or a dye solution such as methylene blue. He gave the general reaction as follows:

$$R'.N{:}N.R'' + 4\,TiCl_3 + 4\,HCl \longrightarrow R'.NH_2 + R''.NH_2 + 4\,TiCl_4$$

Azobenzene and its derivatives are reduced at the dropping mercury electrode in neutral, acid, and alkaline media. Using the polarograph, Tachi (*39*) measured the reduction potentials of azobenzene, *p*-aminoazobenzene, and dimethylaminoazobenzene in dilute ethanol solution at 0.001 *M* concentration.

AMINO NITROGEN ($—NH_2$)

A widely used method applicable to the determination of many aliphatic primary amino groups is that developed by Van Slyke (*40*). The general reaction is:

$$RNH_2 + HNO_2 \longrightarrow ROH + H_2O + N_2$$

The nitrous acid is prepared from sodium nitrite and glacial acetic acid. After the reaction, the gas mixture is treated with alkaline permanganate in a Hempel pipet, and the nitrogen, which remains, is measured in a buret. Since publication of the original paper, a micromanometric apparatus has been developed which permits the determination of much smaller amounts of nitrogen (*41*). A general write-up of the method and description of the apparatus are given by Peters and Van Slyke (*27*). Van Slyke's original paper (*40*) discusses the manner in which various types of aliphatic amines react with nitrous acid. The most significant facts are that the amino groups in the α-amino acids react quantitatively in 3 or 4 minutes at room temperature, whereas amino groups in other types of substances react much more slowly. For example, methylamine and ammonia react completely only after shaking for about 2 hours and aminopurine or aminopyrimidine after 2 to 5 hours. Urea required 8 hours. No nitrogen is split off from the amino group in creatine.

Primary and secondary amines possess active hydrogen, and can therefore be determined by the method of Zerewitinoff (*44*). This procedure depends on the quantitative reaction in a closed system of the sample and Grignard reagent, to yield methane. The reactions are as follows:

$$RNH_2 + 2CH_3MgI \longrightarrow 2CH_4 + RN(MgI)_2$$

$$R_2NH + CH_3MgI \longrightarrow CH_4 + R_2NMgI$$

With primary aromatic amines such as β-naphthylamine and *p*-toluidine, Sudborough and Hibbert (*38*) found that heating at 125° to 130° C. was necessary for complete reaction. Only one volume of methane was evolved from secondary amines such as diphenylamine and ethylaniline. Zerewitinoff analyzed succinimide and phthalimide, and found that one mole of methane was evolved. No reaction takes place with tertiary amines. A detailed write-up of the use of the Zerewitinoff method is given by Niederl and Niederl (*25*) and by Pregl (*30*).

Primary aromatic amines, such as aniline and the toluidines. may also be determined by diazotization in hydrochloric acid solution with a standard solution of sodium nitrite, the end point being detected by using potassium iodide–starch paper as an outside indicator (*1*).

ALKIMIDE NITROGEN ($=NCH_3$)

Alkylimino groups of low molecular weight may be determined in organic compounds by a modification of the Zeisel method. The sample is refluxed with 57% hydriodic acid to form the respective quaternary ammonium iodide. Upon heating at 360° C. the lower alkyl radicals (methyl, ethyl, etc.) are split off from the nitrogen in the form of their alkyl iodides.

Friedrich (*12*) improved the original apparatus, while Vieböck and Brecher (*42*) developed the volumetric procedure for determining the alkyl iodides. Two or more distillations must be made to be certain that all the alkyl iodide has been swept out of the reaction flask.

Samples which are insoluble in hydriodic acid may give low results; the addition of phenol and acetic anhydride to dissolve the sample, then completing the determination, is helpful.

The methylimino group in atropine may be determined by this method. Kuhn and Giral (*20*) analyzed trimethylpentadecabetaine successfully. When more than one group is present, caution must be exercised in interpreting the results, for all the groups may not react. Slotta and Haberland (*37*) analyzed cocaine hydrochloride and codeine, which contain both methoxyl and methylimino groups. The former group was measured by treating with hydriodic acid at 135° C., then changing absorbers, heating at 360° to 380° C., and measuring the methylimino group. A detailed write-up of the method is given by Niederl and Niederl (*25*) and by Pregl (*30*).

MISCELLANEOUS NITROGEN GROUPS

Several types of nitrogen linkage in organic compounds may be determined by the Zerewitinoff method. In pyridine at room temperature, acetamide reacts with Grignard reagent to evolve 1 mole of methane; at 95° C. 2 moles are evolved. Acetanilide evolves 1 mole of methane at room temperature; aniline reacts to give only 1 mole, even on warming. Nitriles and isonitriles react with Grignard reagent without the evolution of methane; however, the amount of reaction can be determined by adding water or aniline and measuring the unconsumed reagent.

Rose and Ziliotto (*32*) developed a modification of the method of Friedrich *et al.* (*13*), whereby the sample is first treated with potassium iodide and sulfuric acid to reduce the nitrile. The determination is then completed by the regular Kjeldahl method. They successfully analyzed several nitrile compounds, including acrylonitrile and benzonitrile.

Using the polarograph, Pech (*26*) quantitatively determined saccharin (*o*-benzoic sulfimide), using a supporting electrolyte that was about 0.05 *N* in hydrochloric acid and 0.05 *N* in potassium chloride. Quinoline and nicotine in dilute solution may also be determined polarographically.

Mitchell and Ashby (*23*) determined unsubstituted acid amides by measuring the increase in acidity on reaction with 3,5-dinitrobenzoyl chloride in pyridine. A blank is run on the reagent plus alcohol. Water interferes, but can be corrected for. The method is generally applicable to primary amides of mono- and dibasic aliphatic and aromatic acids such as acetamide, benzamide, and phthalamide.

SUMMARY

Included in this review paper are literature references and an outline of the procedures for the determination of the nitrate, nitro, diazo, azo, amino, amide, alkimide, and nitrile groups in organic compounds. The methods listed are specific for the particular group in question, but they may not necessarily be the shortest ones to use if no interfering constituents are present.

In applying an apparently appropriate method to the analysis of a new compound, it is always advisable first to apply the method to a known compound whose structure approximates that of the sample to be analyzed.

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Physicochemical Micromethods of Test for Petroleum Products

HARRY LEVIN, A. B. MORRISON, and C. R. REED
The Texas Company, Beacon, N. Y.

Micromethods are described for pour point, titer, and vapor pressure, each of which employs less than 1% of the sample used in conventional macromethods. Comparisons are made between results by the micro- and macromethods.

Because the modern petroleum industry is concerned with highly chemical operations and processes and miscellaneous organic and inorganic materials either as products of its own manufacture or items of purchase relating to its plant operations, microchemical procedures, both analytical and synthetic, are of intense interest and importance. However, miscellaneous physicochemical measurements—some very empirical—are also of great importance to this industry because they are used in control of manufacturing operations, included in customers' specifications, and involved in analysis of samples from service and complaints. Macromethods are generally used, but occasionally the sample is too small; this frequently happens when the case is particularly important. Micromodifications of these macromethods have often proved a godsend.

Methods for determining viscosity on microsamples of oil have been reported by Levin (*7*) and Cannon (*4*) and micromethods for unworked and worked consistency of grease by Kaufman (*6*) and Hain (*5*). The present paper describes methods that have been successfully used on relatively small quantities of sample to determine the pour point of lubricating oil, titer of fatty acids, and vapor pressure of gasoline.

POUR POINT

By a simple modification of the well known practice of determining solidification or melting point of wax on a thermometer bulb, it has been possible on a drop of oil to obtain pour test results which agree very well, generally, with those obtained by the conventional A.S.T.M. method (*1*) involving some 30 or 40 grams of sample. The apparatus is shown in Figure 1.

To make a determination, the bulb of the thermometer at room temperature is dipped into the oil at room temperature, to a depth of about 3 mm. If the drop of oil that adheres appears so large that it will be likely to fall off in subsequent manipulations, it should be reduced. The copper wire (No. 29 gage, 5 cm. long) is bent into a single loop 4 to 6 mm. in diameter at one end at a right angle to its stem, which should be cut to be 25 mm. long. The loop is applied to the drop of oil on the bulb where it adheres by surface tension, and the stem serves to magnify the movement of the drop in the subsequent observations. A vertically multilined label pasted inside the pour test jar facilitates observation of the movement of the wire.

The pour test jar, thermometer, etc., are now assembled in the customary manner and the assembly is preheated and cooled from bath to bath exactly as for an A.S.T.M. pour test. The assembly is removed for observation at each temperature drop of 5° F. (2.8° C.). The maximum period that the assembly may be kept out of the cold bath for an observation is 3 seconds, as in the A.S.T.M. method. The temperature at which no movement is noticeable when the assembly is in a horizontal position for 5 seconds is 5° F. (2.8° C.) below the pour point, as in the A.S.T.M. method. Oils of high pour point may be heated, before application on the thermometer bulb, to the same temperatures permitted in the A.S.T.M. maximum pour method. All manipulations, after the assembly of the apparatus, are in strict accordance with the A.S.T.M. method of test for maximum pour point.

The results obtained by this pour point microprocedure and by the A.S.T.M. (maximum) method on a number of types of oil commonly encountered are shown in Table I. The precision of the micromethod, as well as the agreement between its results and those by the A.S.T.M. method, is within the tolerance of the A.S.T.M. method. The one exception is that of undewaxed cylinder stock having a pour point on the order of 80° F. The authors have tried many conceivable variations of the pour point micromethod, but could not correct the situation with regard to this stock.

The micro pour test has been particularly useful as an aid in establishing the identity of the unaltered residue from used and oxidized lubricating oils, greases, and deposits.

TITER TEST

By still another simple modification, it is possible to obtain good indications of the titer on a single drop of liquefied fatty acids. The apparatus is shown in Figure 2.

The fatty acid is melted on a steam bath and the bulb of the thermometer is dipped into the molten mass, as in the pour point microdetermination. The thermometer is held in a test tube with a cork so that the copper wire is about 12 mm. from the bottom of the tube. With another cork the test tube is fixed in the pour test jar, which contains water at room temperature; the cork has a small slit to permit vapors to escape. The assembly is placed in a beaker of water, at room temperature, which is then heated until the thermometer is about 10° C. above the visible liquefaction point of the acids, and then the source of heat is removed. The pour test jar assembly is removed from the beaker and carefully tilted from the vertical position at intervals of 0.3° C. fall in temperature, and the copper wire is observed for movement. The highest temperature at which there is no movement of the wire is recorded as the approximate titer. The assembly is reheated, as before, to liquefy the acids and the observations on cooling are repeated, but this time the first observation is made at 2° C. above the approximate titer. The highest temperature at which there is no movement of the wire is recorded as the microtiter, uncorrected. This value is corrected by a curve (Figure 3) correlating uncorrected microtiters with macrotiters (*3*) obtained on acids recovered from the fats and greases, shown in Table II.

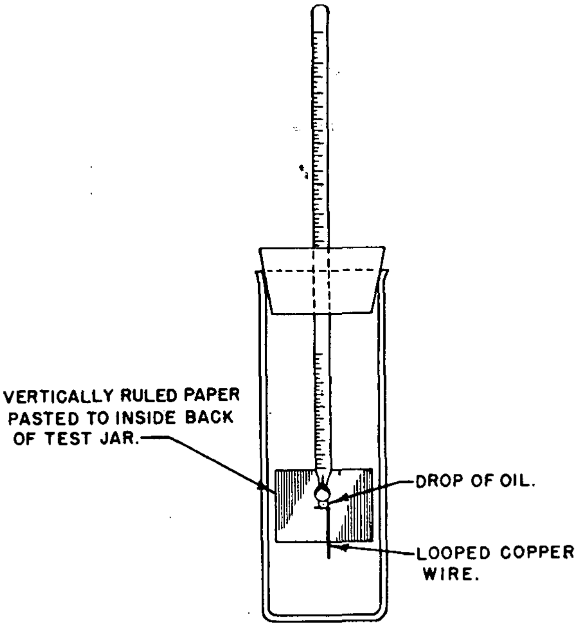

Figure 1. Apparatus for Pour Point Test

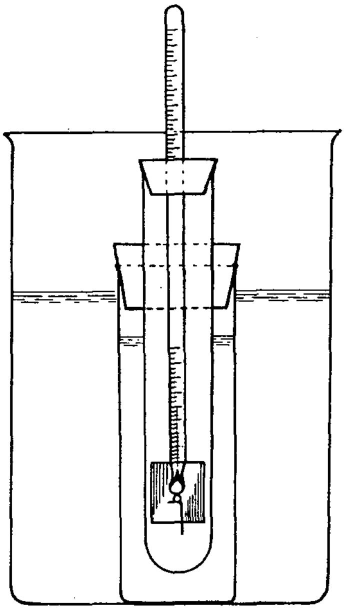

Figure 2. Apparatus for Titer Test

In calibration the corrected value was seldom more than 1° C. away from the macrodetermination. The microtiter is generally reproduced within 0.6° C. The microtiter requires one drop of fatty acids, the macroprocedure some 20 grams.

The micromethod has contributed to the ability to determine if grease left on ball and roller bearings which had "frozen" in storage was the grease intended, when the quantities available were much too small for macroprocedures. The method was likewise uniquely applicable to the analysis of small amounts of grease recovered from bearings on a complaint that the product was too liquid and here, too, the question was one of identity. In each case the microtiter was made on the fatty acids isolated from the soaps in which they were combined.

The authors have used also a method involving a vacuum-jacketed microtube and thermocouple, to obtain a cooling curve simulating the macroprocedure for titer, but the results were no better than those obtained by the much simpler device and procedure that are described in this paper.

Table I. Comparative Data from Pour Point Methods

Sample	Gravity, A.P.I.	Viscosity, S. U.		Viscosity Index	Pour Point, ° F.	
		100° F.	210° F.		A.S.T.M. max.	Micro
Pale oil	27.1	180	..	95	+20 +20	+15 +15
Pale oil + 1% Paraflow	..	..	..	..	−15 −15	−20 −20
Pale oil + 1% Paratone	..	..	..	..	+20 +20	+15 +15
Pale oil	26.7	207	..	84	+95 +95	+95 +90
Pale oil distillate	28.8	85	..	68	+70 +70	+70 +65
Pale oil	23.5	104	..	57	−45 −45	−40 −40
Pale oil	20.4	311	..	0	−25 −20	−20 −15
Pale oil	19.5	761	..	6	− 5 − 5	−5 0
Airplane oil	27.0	..	97	94	+ 5 + 5	0 0
Dewaxed cylinder stock (green)	22.9	..	191	85	+35 +35	+30 +25
Bright cylinder stock (red)	24.7	..	132	85	+10 +15	+ 5 + 5
Undewaxed cylinder stock	22.1	..	161	75	+75 +75	+50 +60
Lard oil	..	..	..	..	+40 +40	+40 +40

VAPOR PRESSURE

An apparatus which has proved very satisfactory for determining vapor pressure on 1 ml. of sample in the gasoline range is shown in Figure 4. In use it is mounted on a stick calibrated in pounds per square inch, the customary system in the petroleum industry.

The apparatus is a manometer (and leveling bulb) made of 8-mm. (outside diameter) glass tubing with a bulb, ap-

Table II. Comparative Data from Titer Methods

Nature or Source of Fatty Acids	Titer Test, ° C. Macromethod	Titer Test, ° C. Micromethod[a]
Neo fat 1-65	67.0	66.5
Palmitic acid	60.1	59.4
Snodotte acids	54.7	53.5
Stearic acid, commercial	52.9	51.4
Neo fat 1-13	50.8	50.0
Myristic acid	48.8	47.2
Cocoa butter	48.2	47.0
Hydrogenated fish oil	46.4	45.0
Commercial lubricating grease	44.5	43.7
Acidless tallow oil	42.1	39.8
Commercial lubricating grease	41.1	39.6
Conduit grease	40.2	38.3
Special lubricating oil	35.6	34.3
25% stearic acid 75% oleic acid	34.2	31.3
Oil from sheep hide	33.8	33.0
Commercial lubricating grease	32.4	31.3
Cottonseed oil	31.1	30
No. 1 lard oil	30.8	28.6
Commercial lubricating grease	26.7	24.8

[a] Direct thermometer reading; not from curve.

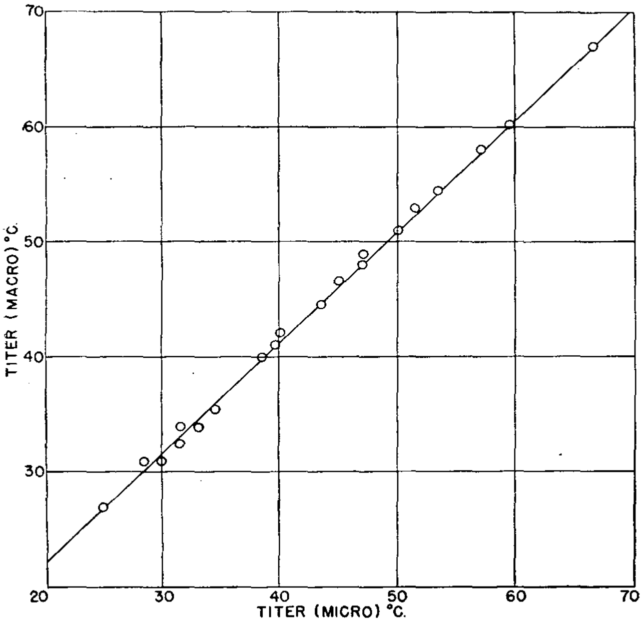

Figure 3. Correlation of Uncorrected Micro- and Macrotiters

proximately 15 cc., blown in the leg equipped with the stopcock. In the authors' apparatus the volume from line *B* to the stopcock is 4.18 cc. and 0.83 ml. of sample is used, thus retaining the A.S.T.M. (*2*) requirement of 4 to 1 vapor to liquid ratio. The volume from *B* to the stopcock was determined by drawing mercury into the inverted apparatus through the stopcock, then weighing the mercury and correcting for the contents of the bore of the stopcock. To introduce the sample a screw plunger micropipet, shown in the figure, is used.

To make a determination the apparatus, with stopcock open and mercury level at *A*, is placed in an ice-water bath for 10 minutes, a rubber cap sealing the open end above the stopcock, to exclude moisture. After about 5 minutes the mercury level is checked and if necessary readjusted (to *A*) by opening the pinchclamp.

Beginning with the mercury level at *A* eliminates the need for correcting for the change in air volume on going from ice temperature to 100° F. The position of *A* is established by bringing the mercury level to *B* at 100° F. and adding, as sample, Octoil S, closing the stopcock, and bringing the assembly to ice temperature. The new position of the mercury surface at this temperature is *A*.

The vapor pressure of Octoil S (Distillation Products, Inc., Rochester, N. Y.) at 100° F. is practically zero.

With suitable precautions to exclude moisture, the pipet at a temperature of 32° to 40° F. (0° to 4.4° C.) is filled to the mark with sample at the same temperature, the sample is introduced to the apparatus through the bore of the stopcock, and the stopcock is closed. By lowering the leveling bulb a small *negative* pressure is imposed on the 15-cc. bulb. This effects equilibrium faster in subsequent operations. However, if the vapor pressure is relatively high, it may be necessary to raise the leveling bulb and impose a positive pressure to avoid loss of sample from the bottom of the 15-cc. bulb through the left arm of the manometer, during subsequent shaking. It will be obvious when pressurizing is necessary.

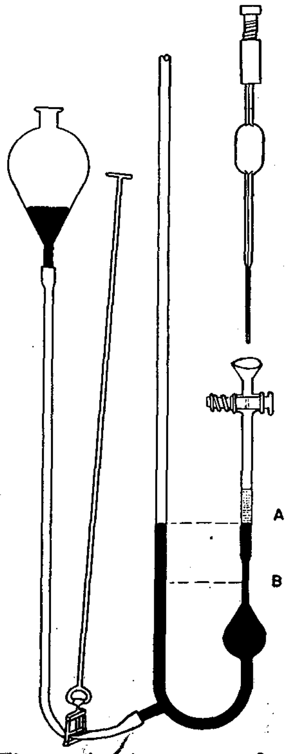

Figure 4. Apparatus for Determining Vapor Pressure

The apparatus is then put in a water bath at 100° F. (37.7° C.), and the rubber cap of an eyedropper is placed over the funnel-shaped outlet above the stopcock to keep water out of the apparatus. After 5 minutes the apparatus, exclusive of the leveling bulb, is shaken for about a minute without being removed from the water bath. The mercury level is returned to *B* by opening the pinchcock, which is then closed, and the pressure is read directly on the manometer. Several successive readings are taken to ensure accuracy.

To prepare for the next sample, the apparatus is taken from the bath, the rubber cap is removed, the stopcock is opened, the mercury level is raised into the opening above the stopcock, and the tested sample is removed with an eyedropper. It is advisable to remove also about 1 ml. of the mercury, in the same manner, to eliminate contamination by reaction products which tend to emulsify the surface layer of mercury and make precise level readings difficult. The mercury leveling bulb is lowered and raised several times to remove last traces of old sample by aerating the sample tube. The apparatus is then ready for a new sample. The elapsed time for a determination is about 30 minutes, during which several repeat readings are obtained on the contents of the microapparatus.

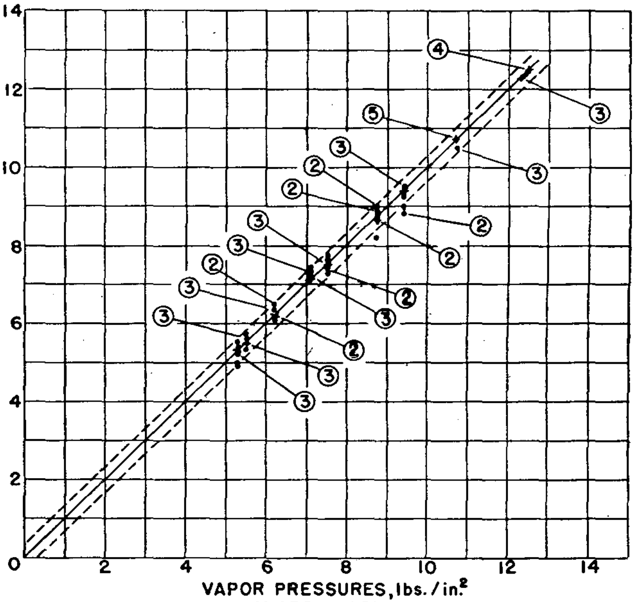

Figure 5. Comparative Results by Macro- and Micromethods

Comparative results obtained on gasolines by the micromethod on 1-ml. samples and by the A.S.T.M. method requiring 130-ml. samples are shown in Figure 5. The solid line is A.S.T.M. vapor pressure, located by points *X;* the dots are microvalues and the numbers at the dots represent the numbers of identical results obtained for the points shown, about half of them being repeats on one filling, and the rest individual tests on separate fillings. In practically every case the micro result is within 0.2 pound of the A.S.T.M. value. The standard deviation of the micromethod is 0.2 pound.

This apparatus and technique can probably be adapted to even smaller samples. The vapor pressure microprocedure is useful in carburetor studies, test tube synthesis, stability studies, etc.

Natelson (*8*) described an ingenious method for determining vapor pressure on a single drop of liquid. Unfortunately, it is limited by its authors to pure substances and the present authors' experience confirms its inapplicability to a mixture of hydrocarbons like gasoline. The present method is, of course, applicable also to pure hydrocarbons in the gasoline range.

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NOTES ON ANALYTICAL PROCEDURES . . .

Manometric Apparatus for Gas Measurements on Packaged Materials

J. L. BLATT AND N. P. TARASSUK
Dairy Industry Division, University of California, Davis, Calif.

IN RECENT years, there has been an increasing interest in the relation of quality deterioration of various food and industrial products to the nature of the atmosphere surrounding the material during its processing or storage (*1–5, 7–9*). Some useful correlations have been made simply by the analysis of gases taken from containers before and after the material has been subjected to test conditions. However, in order to correlate certain quality changes with absolute gas pressures, or with milliliters (S.T.P.) of atmospheric gas per gram of packaged material, it is necessary also to determine the effective volume and pressure of the confined atmosphere, and it is preferable, though usually not possible with most equipment, to make all measurements in a continuous integrated procedure.

Cartwright (*3*) has described a noteworthy apparatus which, in addition to permitting the determination of quality and quantity of gases about dry materials in all manner of containers, is adaptable to the detection of leakage in dry vacuumized packages, to the determination of gas permeabilities of packaging materials, to the determination of densities of dry irregular substances, and to the estimation of air entrapped in powders during manufacture.

A portion of gas from a test container is transferred into a buret evacuated with a mechanical pump. The volume is read following compression under mercury to atmospheric pressure. By using a leveling bulb and manipulating three of the system's five stopcocks, this gas is expelled and a similar fraction is introduced and measured. The original volume and pressure of gases in the container are derived from these values. The measurement of barometric pressure is determined separately. Samples of gas for analysis may be withdrawn from the container and transferred to an appropriate system. Fair reproducibility and accuracy are demonstrated when the packaged materials are essentially "dry" and relatively nondesorbing of gases during the procedure.

In the use of a simplified manometric apparatus the limitations of moisture and desorption inherent in Cartwright's method are largely overcome, and distinct advantages in simplicity of construction and operation and precision in measurement are gained. In this procedure, the gas pressure and free space volume in a container are derived from the measurement of pressure when the volume of the container gases is expanded by two fixed amounts. If

V_x = an unknown volume (the effective free space in a container)
P_x = original gas pressure in the container
V_a = volume of one expansion
V_b = volume of another expansion
P_1 = pressure of gases after expansion into V_a—i.e., pressure at volume $V_1 = (V_x + V_a)$
P_2 = pressure of gases after expansion into V_b—i.e., pressure at volume $V_2 = (V_x + V_b)$

and the temperature and the number of gas molecules measured remain constant,

$$P_xV_x = K = P_1V_1 = P_1(V_x + V_a) = P_2V_2 = P_2(V_x + V_b)$$

from which

$$V_x = \frac{P_2V_b - P_1V_a}{P_1 - P_2}$$

or preferably

$$V_x = \frac{V_a - V_b(P_2/P_1)}{P_2/P_1 - 1}$$

and

$$P_x = \frac{P_1(V_x + V_a)}{V_x} = \frac{P_2(V_x + V_b)}{V_x}$$

Once the P_2/P_1 ratio is found, graphs or tables may be constructed to eliminate the individual calculations of V_x and the pressure factor $\left(\frac{V_x + V_a}{V_x}\right)$, inasmuch as V_a and V_b are constants.

Components which of necessity are common to both manometric and volumetric systems are a puncturing device, a means for clearing the system of contaminating gases before measurements are made, a measuring chamber into which container gases are expanded, a leveling system for the transfer and compression of gases, and a communicating passage to a gas analyzing system.

These features have been met by a simple structure (illustrated in Figure 1) which combines a punch, *P*, a three-way 120° capillary bore stopcock, *C*, an expansion bulb, *E*, a 30-inch open Y manometer tube, *M*, an overflow trap, *O*, a leveling bulb, *L*, and an adjustable scale, *S*, all mounted on a wood and metal supporting structure, *D* and *F*, such that most operations and observations are made at eye level when one is standing.

The glass components are relatively easily fashioned from stock Pyrex parts, the Y-tube from small-bore heavy-walled stock, and the leveling bulb, the overflow bulb, and expansion bulb from round-bottomed flasks. Translucent rubber is used for connecting passages E_1 and E_2, which are made as short as practical. Scale *S* is a meter stick, calibrated in millimeters from bottom to top, and fitted immediately along side the left-hand manometer tube, so that it can be moved vertically through a distance sufficient to cover the range of local barometric pressures. It is held at a desired position with a wing nut and bolt. A sliding leveling bulb holder, *H*, allows the bulb to be adjusted to and held at any desired height. The punch crank assembly, *K*, the sampling platform, *A*, the manometer board, *D*, the leveling bulb slide bar, *Z*, and other accessories are mounted to the pipe-supporting frame, *F*, by movable 90° double-grip clamps, *G*.

Permanent reference levels *b* and *a* are placed one above, the other below the level of bulb *E*, and the volumes enclosed between the surface of the container at *P* and each of these levels in the chamber are calibrated—e.g., by using known container volumes. In the authors' apparatus, these volumes (V_b and V_a) are 3.0 and 150.0 ml., respectively.

COMPARATIVE FEATURES

Advantages in this apparatus arise from (1) features that could apply equally well to volume measurement techniques, and (2) those that result from measuring pressure rather than volume.

Simplification of Stopcock System. The introduction and expelling of gases through a single three-way port at the top of the measuring chamber not only facilitates the handling of gases with the leveling bulb, but (in reducing the number of stopcocks so needed from three to one) it simplifies construction and considerably lessens the maintenance needed to prevent leaks.

Taking Measurements in a System Saturated with Water Vapor. By maintaining moisture on the walls of the measuring chamber, the behavior of water vapor in gas samples is changed from an unknown to a predictable and controllable factor. (If water is drawn into the measuring chamber and expelled, sufficient moisture remains on the walls to keep the chamber saturated with water vapor until cleaning is required.) In this technique, the contribution of water vapor to the total pressure is automatically deducted in the scale setting. (With the moist chamber evacuated, and the mercury in the chamber set at reference level *a*, the zero of the adjustable scale is set opposite the mercury level in the left manometer tube. Thereafter, the "dry" pressure of gases held at level *a* in the chamber can be read directly from the scale, regardless of whether gases in the container were moist or dry.) In volume measurements the barometric pressure used in calculations would in each case be effectively reduced by a fixed amount—i.e., by saturated vapor pressure.

Elimination of Mechanical Pump. Instead of a mechanical pump, the leveling system can be used as a piston in clearing the measuring chamber and connecting passages of interfering gases by expelling them through E_2. This double use of the leveling system not only eliminates the pump and its stopcock but allows the retention of moisture in the chamber.

Replacing Volumetric Buret by Manometer Tubes and a Linear Scale. This simplifies construction and readings. Measurements in this case are of length rather than volume; hence, many inexpensive scales, whose calibrations are easily and precisely checked, can be purchased, from which readings of three- and four-place figures can be made without the necessity of applying corrections. The readings themselves are made by bringing mercury to fixed levels in capillary tubing rather than by matching mercury columns of relatively large diameters.

Reducing Inducement of Materials to "Desorb." As it is necessary neither to remove gas from the measuring chamber nor to manipulate stopcocks before making a second required reading but only to change the mercury level, measurement is concluded quickly and with a minimum of exposure of materials to altered pressures. The volume technique necessarily exposes material during the period of the first measurement to pressure changes of considerably greater magnitude and for longer duration.

Elimination of Barometer Readings and Vapor Pressure Values in Calculations. Although the height of mercury sustained in an "evacuated" chamber is a function of both barometric pressure and the magnitude of saturated vapor pressure, measurement of these values is eliminated in the scale setting

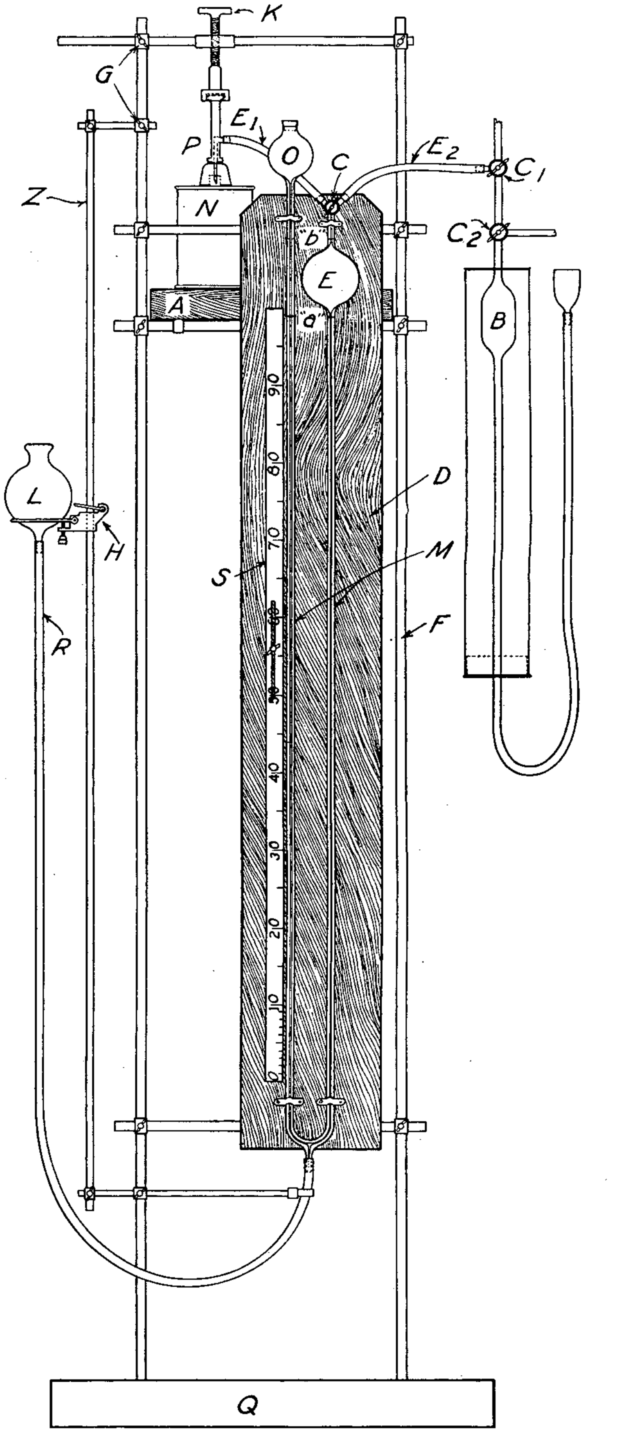

Figure 1. Apparatus

and does not enter into calculations. In volume measurements, calculations must take into account the value of barometric pressure and the magnitude of saturated vapor pressure (or some value of vapor pressure) in addition to the value of atmospheric temperature utilized by both methods in converting figures to a common base.

OPERATIONAL PROCEDURE

Union of container *N* with the measuring apparatus is made by moistening the surface of the hollow rubber pad which surrounds punch *P*, and then compressing the pad by turning the pressure crank, *K*, sufficiently to make a gas-tight bond.

In evacuating the system with the leveling bulb, gases in *E* are first expelled through E_2; thereafter, most of the gas in connecting passage E_1 can be expanded into *E* and then expelled. Usually a single repetition of the procedure is sufficient for the "complete" evacuation, which is seen visually from the scale when the height of mercury sustained in the evacuated chamber by atmospheric pressure is the same for the whole system as for chamber *E* alone. Leaks into the vacuumized system can be very quickly and critically detected (as evidenced by an ebbing mercury column) in setting the mercury at mark *b*. After evacuating, a mercury seal can be left in passage E_2 as an added precaution against leakage.

The adjustable scale may be set for the atmospheric temperature and pressure conditions peculiar to each determination.

The container is punctured by further turning the pressure crank, *K;* by using the leveling bulb the confining volume is successively extended to marks *b* and *a*, and the resultant pressures, P_2 and P_1, are noted. For the pressure of gases held at the upper mark, *b*, to avoid moving the scale, the vertical distance between marks *a* and *b* is subtracted from the corresponding direct scale reading. For convenience in reading, this distance is made to be an exact 100 mm.

The reproducibility to be expected in readings, the validity of the scale setting, and the successful control of water vapor in pressure measurements are demonstrated in Table I by the constancy of the several P_2/P_1 values obtained with different amounts of moist gas in a system of fixed volume.

Gas samples for analysis may be withdrawn from the container to any extent desired by using the leveling bulb and transferred to the analyzer buret, *B*, in the amounts needed. The relative pressure of gases in the chamber is immediately apparent from the manometer scale. When it is desirable to conserve test gas, the passageway to the buret, E_2, may be cleared by displacement with mercury, obviating the need for rinsing or sweeping. By virtue of its simplicity, the apparatus is conveniently used as a sampling and transfer device, even when volume and pressure measurements are not required.

Table I. Reproducibility of P_2/P_1 Ratio with Varying Amounts of Moist Gas in System of Fixed Volume

Trial	P_2	P_1	Ratio	Dev. from Mean
1	754.0	482.0	1.5643	0.0004
2	711.0	454.0	1.5661	0.0022
3	607.0	389.0	1.5604	0.0035
4	540.0	345.0	1.5650	0.0011
5	491.0	314.0	1.5636	0.0003
		Mean	1.5639	0.0015

ACCURACY

Representative calculations incorporating gas analysis data are illustrated in Table II for determinations made on a container of powdered ice cream mix originally sealed in air, and another on nonfat dry milk solids originally sealed in an atmosphere of nitrogen. The figures given are particularly significant, not in regard to the quality of the materials and the packaging (as there are many variables not indicated), but as illustrative of the procedure and precision of results to be expected.

The estimated probable error for each step in the determination is separately indicated for the first example. In quantities such as P_1 and P_2 which are measured directly, errors given are determined largely by the specifications and calibration of equipment, and by the reproducibility of measurements. Estimations of the error in calculated quantities, such as V_x and P_x, were derived by a method of partial differentiation (*6*) of the expressions from which these values were calculated and from the probable errors of the quantities measured directly. In the example cited in Table II, the partial errors in V_x due to the probable errors in measurement of P_2/P_1, V_a and V_b were calculated as 1.9, 1.4, and 1.9 ml., respectively, the most probable error (indicated in the table) due to their combined effects being 3.1 ml.

The sensitivities and accuracies indicated for the determinations of V_x, P_x, density, and the milliliters of gas in this method compare well with similar measurements made by other means, and might be improved in some cases by refinement in specifications and calibration. For example, from the expression for the derivation of V_x it is seen that the sensitivity of the determination is dependent on the structural specifications of the apparatus—i.e., on the size of $\frac{V_x + V_a}{V_x + V_b}$ which determines the ratio of P_2/P_1. With the limitations indicated for the chamber volume calibrations and for the reproducibility of pressure readings, the capacity of bulb *E* needs to be approximately 10% or more of the volume of V_x, and the passageway volume E_1 kept small to get full accuracy from the apparatus.

Table II. Representative Determinations Illustrating Calculations and Precision of Measurement

Determination	Quantity	Derivation	Example 1 (Ice Cream Mix)	Probable Error	Example 2 (Milk Powder)
Pressure and free space	P_2	Meas.	694.0 mm.	±0.5	733.0 mm.
	P_1	Meas.	513.0 mm.	±0.5	470.0 mm.
	P_2/P_1	Calcd.	1.353	±0.0016	1.560
	V_x	$\frac{V_a - V_b \times (P_2/P_1)^a}{P_2/P_1 - 1}$	422 ml.	±3.1	265 ml.
	P_x	$\frac{P_1(V_x + V_a)}{V_x}$	699 mm.	±1.6	741 mm.
Apparent density[b]	W_m (wt. of material)	Meas.	454 grams	±3.0	241 grams
	V_c (vol. of container)	Meas.	835 ml.	±6.0	452 ml.
	V_m (vol. of material)	$V_c - V_x$	413 ml.	±6.8	187 ml.
	Apparent density	W_m/V_m	1.10	±0.018	1.29
Temperature of measurements	$T°$	Meas.	25.6° C.	±0.2	23 2° C.
Haldane analysis	CO_2	Meas.	2.04%	±0.05	1.14%
	O_2	Meas.	9.71%	±0.05	0.11%
Partial pressure at 0° C.	CO_2	$\frac{\% \ CO_2 \times P_x \times 273}{100\ (T + 273)}$	13.0 mm.	±0.32	7.78 mm.
	O_2	$\frac{\% \ O_2 \times P_x \times 273}{100\ (T + 273)}$	62.0 mm.	±0.35	0.75 mm.
Gas, ml. (S.T.P.) per gram of material	CO_2	$\frac{V_x \times P_{CO_2}}{760 \times W_m}$	0.0159 ml./gram	±0.0004	0.0112 ml./gram
	O_2	$\frac{V_x \times P_{O_2}}{760 \times W_m}$	0.0758 ml./gram	±0.0009	0.0011 ml./gram

[a] V_a (calibrated) = 153.0 ± 0.5 ml.
V_b (calibrated) = 3.0 ± 0.5 ml.

[b] Designates density of milk solids together with air cells entrapped in powder during manufacture, as contrasted with "cell-free" density.

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Determination of Phosphorus in Iron Ore

JAMES L. KASSNER and MARY ALICE OZIER

University of Alabama, University, Ala.

THE determination of phosphorus in iron ore is carried out in three steps: solution of the sample, precipitation of the ammonium molybdiphosphate (*7*), and alkalimetric titration of this salt to determine the amount of phosphorus present. This study deals specifically with the second and third steps in this procedure. The solution of the sample is effected by standard procedure.

The single-strength citromolybdate solution used in the determination of phosphorus pentoxide in phosphate rock (*3*) did not give complete precipitation when used in the determination of phosphorus in iron ore. After considerable experimentation a double-strength citromolybdate solution was prepared and found to be stable. This study has shown that the time required for a complete determination of phosphorus in iron ore can be shortened by using the new double-strength citromolybdate solution and separating the ammonium molybdiphosphate at the boiling point. The analysis of several Bureau of Standards samples indicates that the precision and accuracy of the method are good.

The new mixed indicator (*3*) used in the determination of phosphorus pentoxide in phosphate rock is used in determining the end point.

REAGENTS AND STANDARD SOLUTIONS

Double-Strength Citromolybdate Solution. Solution A. Dissolve the following reagents in 1400 ml. of water and warm while stirring until solution is complete: 100 grams of ammonium nitrate, 128 grams of citric acid monohydrate, and 136 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$.

Solution B. Dilute 528 ml. of concentrated nitric acid (specific gravity 1.42) with 300 ml. of water.

Prepare the citromolybdate solution by pouring Solution A into Solution B. Clear it as follows: Add 10 to 15 drops of 20% diammonium hydrogen phosphate solution, boil 5 to 10 minutes, allow to stand overnight; then siphon off the clear solution. (Such a solution remained clear 2 years.)

The other reagents for this method are essentially those used in the determination of phosphorus pentoxide in phosphate rock (*3*).

PROCEDURE

Weigh out a 2-gram sample of iron ore into a 150-ml. beaker, add 20 ml. of concentrated hydrochloric acid (specific gravity 1.19) (*8*), heat the covered solution on a hot plate until the ore is dissolved, add 5 to 10 ml. of concentrated nitric acid (specific gravity 1.42) (*4*), add 12 to 15 ml. of 60 to 70% perchloric acid (*1, 2, 11, 12*), heat on a hot plate to copious fumes of perchloric acid, and fume at least 5 minutes to dehydrate the silica. Wash the filter with hot 1% nitric acid solution and then with hot water. Reserve the filtrate.

In order to recover any phosphorus that might be retained in the residue, volatilize the silica by treating the residue with an excess of hydrofluoric acid and a small amount of nitric acid (*6*); fuse the residue with sodium carbonate, leach with warm water, and filter to remove any titanium that might be present. Add to the filtrate from the fusion a little ferric chloride free from phosphorus and precipitate the ferric phosphate with ammonia (*10*). Wash the precipitate with hot water, dissolve in not more than 20 ml. of hot 1 to 2 nitric acid solution, and add it to the reserved filtrate.

Dilute the solution to 80 ml., add 100 ml. of double-strength citromolybdate solution, heat to boiling, and keep at this temperature for 5 to 10 minutes. Remove the solution from the heat and filter immediately. Before transferring the precipitate to a Gooch crucible, wash it three times by decantation with 1% nitric acid solution, using about 5 ml. for each wash. Transfer the precipitate to the crucible and wash it 10 to 12 times with 1% potassium nitrate solution. Place the crucible in the original beaker and dissolve the precipitate in a known volume of 0.1 *N* sodium hydroxide solution, using about 30 ml. in excess (*3*). Add 0.5 ml. of the mixed indicator (*3*) and titrate the solution with 0.1 *N* nitric acid until it turns yellow. Remove the crucible from the beaker, wash with carbon dioxide–free water, and adjust the volume to about 100 ml. In direct sunlight or in front of an illuminator equipped with a fluorescent daylight Mazda lamp, back-titrate the solution with 0.1 *N* sodium hydroxide until a purple coloration appears and remains.

The percentage of phosphorus is calculated on the basis of: 1 P ≈ 23 NaOH. Data are tabulated in Table I.

DISCUSSION AND NOTES ON PROCEDURE

Arsenic seems to interfere more in this procedure than it does when the precipitation is carried out at a lower temperature by shaking. If arsenic is present, it should be removed as recommended by Lundell, Hoffman, and Bright (*5*).

Table I. Results Obtained with Bureau of Standards Iron Ores

Sample No.	Interferences Present		P_2O_5 Certified Value	P_2O_5 Exptl. Value	Deviation
	TiO_2	V_2O_5			
	%	%	%	%	
26	0.07		0.09	0.11[a]	+0.02
				0.10[a]	+0.01
				0.10[b]	+0.01
				0.09[b]	0.00
				0.10[c]	+0.01
				0.09[c]	0.00
27B	0.023	0.004[d]	0.036[e]	0.035[a]	−0.001
				0.035[a]	−0.001
				0.031[b]	−0.005
				0.036[b]	0.000
				0.037[c]	+0.001
				0.035[c]	−0.001
29	0.99	0.08	1.01	1.05[a]	+0.04
				1.04[a]	+0.03
				1.05[b]	+0.04
				1.04[b]	+0.03
				1.03[c]	+0.02
				1.04[c]	+0.03
29a	0.15	0.002	0.007	0.008[a]	+0.001
				0.007[a]	0.000
				0.007[b]	0.000
				0.008[b]	+0.001
				0.008[c]	+0.001
				0.007[c]	0.000

[a] Boiled 10 minutes and allowed to stand overnight before filtering.
[b] Boiled 10 minutes and filtered immediately.
[c] Boiled 5 minutes and filtered immediately.
[d] Per cent vanadium.
[e] Per cent phosphorus.

In routine analysis of iron ore at the mine, any phosphorus retained in the acid-insoluble material is often neglected (*9*). If titanium is not present this fraction seldom contains more than 0.003% phosphorus (*6*). The treatment of the sample should be varied according to whether the analysis is being made for soluble, soluble and insoluble, or total phosphorus.

Because iron retards the separation of the yellow precipitate and forms a complex with citric acid, it is necessary to use a more concentrated citromolybdate solution than that used in the determination of phosphorus pentoxide in phosphate rock. Although this solution is referred to as double-strength, it contains more than twice the amount of citric acid used in the original solution (*3*). The double-strength citromolybdate solution must be cleared by adding diammonium phosphate and boiling in order to saturate the solution with the yellow precipitate and to remove colloidal molybdic acid.

The sharpness of the end point can be increased by filtering off the asbestos after making the solution acid and washing it with carbon dioxide–free water. If enough excess of sodium hydroxide is used when dissolving the precipitate (*3*), the end point will be sharp and remain indefinitely. If too little alkali is used in dissolving the precipitate, the end point will come too soon and fade repeatedly.

SUMMARY

Free molybdic acid does not separate at the boiling point when a molybdate solution containing nitric and citric acids of a prescribed composition is used. At the boiling point the yellow precipitate separates almost immediately. The accuracy and precision of this method are good.

ACKNOWLEDGMENT

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Elimination of Interference by Copper in the Titan Yellow Method for Magnesium

A. E. WILLSON and I. W. WANDER
Citrus Experiment Station, Lake Alfred, Fla.

MODIFICATIONS of Kolthoff's titan yellow method (*2*) for determining magnesium are being used extensively for the analysis of soils and plant tissues (*1*). Using a method proposed by Peech and English (*5*), erratic results were obtained on sandy soils planted to citrus in Florida; no magnesium was found in some samples where it was known to be present.

It has been reported that copper ions interfere with the titan yellow procedure (*4*). This effect of copper in a soil extracting solution (*3*) on the determination of magnesium by the Peech and English method results in very low recoveries, as shown in Table I, A.

The addition of cyanide to a soluton containing copper ions results in a soluble copper complex with a dissociation constant of 5×10^{-28}, thus effectively eliminating copper ions from the solution. The effect of potassium cyanide on the copper interference is shown in Table I, B. Variation in the amount of potassium cyanide from 0.05 to 0.8 ml. did not affect the magnesium recovery. The presence of 0.1 ml. of the 5% potassium cyanide solution was able to eliminate the effect up to 40 micrograms of copper satisfactorily (Table I, C).

Because potassium cyanide alone or potassium cyanide with copper has a tendency to intensify the magnesium titan yellow color slightly, it has been found advisable to add copper and potassium cyanide to the magnesium standards. Inasmuch as this effect of copper does not increase appreciably over 8 micrograms, it is suggested that 8 micrograms be added to the standards. Thus in the modified procedure both copper and potassium cyanide are added to the standards and 0.1 ml. of 5% potassium cyanide is added to all unknown.

Inasmuch as the use of copper is recommended in citrus fertilizers, enough copper is present in most soil extracts to prevent reliable estimations of magnesium by titan yellow, as shown in Table II.

Samples 595 and 596 were from areas receiving no copper on the soil. The results given in Table II also show that, with the addition of potassium cyanide to the titan yellow procedure, determinations comparable to an accepted procedure (*6*) can be made.

Table I. Effect of Varying Amounts of Copper and 5% Potassium Cyanide on Magnesium Recovery

(15 micrograms determined in 5 ml. of solution)

	Copper Present, γ	5% Potassium Cyanide, Ml.	Magnesium Recovery, %
A	0	0.0	100
	1	0.0	80
	2	0.0	57
	4	0.0	33
	8	0.0	30
	16	0.0	30
	32	0.0	25
B	16	0.05	107
	16	0.10	100
	16	0.20	99
	16	0.40	100
	16	0.80	105
C	0	0.10	100
	16	0.10	100
	24	0.10	104
	32	0.10	104
	40	0.10	107

Table II. Analysis of Soil Samples by Titan Yellow and Oxine (*6*) Procedures

(Micrograms of magnesium per gram of soil)

Soil Sample	Titan Yellow, No Potassium Cyanide	Titan Yellow + Potassium Cyanide	Oxine Procedure
593	5.7	13.7	12.6
594	2.5	6.0	6.0
595	4.2	4.0	5.9
596	3.0	3.4	5.9
597	2.2	4.7	6.3
598	5.0	14.3	14.7

The copper spray residues on leaf samples may result in sufficient copper in sample solutions to interfere with the titan yellow magnesium procedure. It is therefore advisable to modify such procedures for tissue analyses in a manner similar to that suggested for soils.

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Determination of Fluorine in Organic Compounds by Titration with Aluminum Chloride

MARY JO GILBERT and JOHN H. SAYLOR
Duke University, Durham, N. C.

A PROCEDURE has been described (*4*) for the titration of fluoride ion in a nearly neutral solution with aluminum chloride using Eriochromcyanine R as an indicator. This method has been applied with certain modifications to the determination of fluorine in organic compounds.

ANALYTICAL PROCEDURE

Decompositon of Sample. Efforts to decompose the organic compounds with potassium in sealed glass tubes as described by Elving and Ligett (*1*) usually gave high results for the fluorine content, as previously noted by Kimball and Tufts (*3*). After a trial of several methods of decomposition, the method finally adopted is essentially that described in detail by Kimball and Tufts (*3*), which consists of a decomposition by means of metallic potassium in a nickel bomb at 500° to 550° C.

The nickel bombs used in the present investigation have a small circular groove cut 5 mm. from the outer edge of the cup, which seems to improve the seal between the cup and the lid. In other respects the design of the bomb and its use in the decomposition are as described by Kimball and Tufts.

Solid and high boiling liquid samples are weighed in gelatin capsules. Volatile liquids are weighed in thin-walled glass ampoules, and gases are vacuum-distilled into drawn-out sections of 8-mm. Pyrex tubing, one end of which is cooled by liquid air. The sample size usually should be 0.1 to 0.2 gram.

After the bomb is cooled and opened, the nickel cover is placed in a 50-ml. beaker containing about 2 ml. of purified methanol. The cup is inserted into the reflux apparatus described by Kimball and Tufts and the excess potassium is decomposed by refluxing with methanol. It was not found necessary to purge the apparatus with carbon dioxide.

After the decomposition of the potassium, the reflux cylinder, the nickel cup, the beaker containing the cover, and the cover are rinsed with warm water into an 800-ml. beaker with the aid of a rubber policeman to loosen the carbonaceous material. The final volume is about 400 ml. Then approximately 1 gram of ammonium bicarbonate and a few glass beads are added; the beaker is placed on an asbestos board and the solution is evaporated almost to dryness over a Meker burner. The residue is filtered with the aid of warm water into a 110-ml. flask. The alkaline fluoride solution should be clear and colorless.

Distillation of Decomposition Products. It is necessary to separate the fluoride ion from interfering substances by distillation as hydrofluosilicic acid according to the method of Willard and Winter (*5*). Several types of distillation apparatus described in the literature were tried, but entirely satisfactory results were obtained only with the constant temperature steam-distillation apparatus of Huckabay, Welch, and Metler (*2*). The temperature is maintained constant in this apparatus by refluxing *sym*-tetrachloroethane. Certain minor modifications were made in the original design.

The sulfuric acid and the sample are introduced into the distilling bulb through a long-stemmed funnel fitted with a stopcock and sealed into the stopper of the apparatus. The steam generator is heated on a hot plate and the precise control of the steam flow (which is important) is made possible by inserting a steam trap made from a large adapter between the generator and the distillation apparatus with a screw clamp on the drain tube to control the steam flow. The apparatus is heated with a 200-ml. capacity Glas-col mantle controlled by a Variac.

After steam is being generated, a 10 to 25-ml. aliquot of the sample solution and 2 ml. of 33% silver sulfate are added through the funnel in the top of the distillation apparatus. The silver sulfate may be omitted if no chloride ion is present. Then 20 ml. of 18 M sulfuric acid are added dropwise. While adding the sulfuric acid, heating the distillation flask is started. When the acid has been introduced, a small stream of steam is passed through the solution to mix it and as the tetrachloroethane begins to reflux, the steam flow is increased until approximately 40 drops per minute of distillate are being collected. Three 110-ml. portions of distillate are collected. If the third 110-ml. portion collected does not constitute a blank, 110 ml. more are collected.

When the distillation is completed, the Glas-col heater is turned off, the distilling bulb is emptied, with the use of suction, steam is passed through the apparatus for several minutes, and finally, after cooling to about 50° C., it is thoroughly rinsed with water.

Determination of Fluoride. The procedures for the preparation of the indicator solution, the 0.2 to 0.25 *N* aluminum chloride solution, and its standardization against pure sodium fluoride have been described (*4*).

Better results are obtained when the 0.1% solution of Eriochromcyanine R is not used until it has stood for 4 days and is not used after 14 days. All titrations are done in a darkened room using a Fisher illuminator containing a daylight fluorescent lamp. All volumetric apparatus is calibrated.

Two 50-ml. aliquots are withdrawn from each of the 110-ml. volumetric flasks containing the distillate and are placed in 125-ml. Erlenmeyer flasks. Then to each aliquot are added 25 grams of sodium chloride (reagent grade, sulfate-free) and 2 drops of phenolphthalein. The solution is made slightly basic with 0.5 *N* or 0.1 *N* sodium hydroxide, depending on the amount of the hydrofluosilicic acid in the distillate and heated to 90° to 100° C. on a hot plate, and the pH of the solution is carefully adjusted with split drops of 0.1 *N* hydrochloric acid added from a dropper with a capillary tip until the pink color of the phenolphthalein just disappears. It is important to avoid a large initial excess of sodium hydroxide, as such an excess leaves the solution with a yellow tinge after the final adjustment of the pH.

To this colorless and slightly boiling solution are added 10 drops (1 drop per 5 ml. of solution) of 0.1% Eriochromcyanine R solu-

Table I. Analysis of Organic Fluorine Compounds

Compound	No. of Detns.	% F⁻, Theory	% F⁻, Found
4,5-Dichloro-1,3(trifluoromethyl)benzene, $C_8H_2Cl_2F_6$	5	40.28	40.3 ± 0.2
Benzotrifluoride, $C_7H_5F_3$	3	39.01	39.1 ± 0.1
$C_2H_2OF_2$	2	47.48	47.6 ± 0.1
$C_3H_2Cl_2F_2Br_2$	2	12.39	12.6 ± 0.1
C_4HF_7O	3	67.16	67.0 ± 0.1
$C_4H_2F_6O$	2	63.31	63.5 ± 0.05
C_4F_8O	2	70.36	70.4 ± 0.1
$C_4H_6O_2F_2$	3	30.62	30.6 ± 0.03
C_5F_8O	2	66.65	66.7 ± 0.2
$C_7H_3F_3BrCl$	4	21.97	21.9 ± 0.1
$C_7H_6F_2$	3	29.66	29.6 ± 0.2
$C_7H_7F_7$	2	59.37	59.6 ± 0.05
$C_8H_2F_{14}$	2	73.26	73.3 ± 0.05
$C_8H_2F_{12}$	2	69.92	70.0 ± 0.05
$C_8H_5F_{11}$	2	67.39	67.4 ± 0.0
$C_8H_8F_8$	2	59.34	59.5 ± 0.0
$C_8H_{10}F_6$	2	51.78	51.8 ± 0.05
$C_8H_{13}O_2BF_2$	2	20.00	19.9 ± 0.2
$C_{13}H_9F_3$	4	25.65	25.5 ± 0.04
$C_{13}H_9N_2O_2F_3$	3	20.20	20.4 ± 0.2
$C_{14}H_6N_2O_4F_6$	2	30.00	30.4 ± 0.1
$C_{14}H_8F_6$	5	39.28	39.2 ± 0.3
$C_{16}H_4F_{12}N_2O_4$	2	44.17	44.3 ± 0.1
$C_{20}H_{13}F$	3	6.98	6.8 ± 0.3
$C_{20}H_{15}FO$	2	6.54	6.6 ± 0.2

tion, which gives a slight yellow color to the solution. Then the standard aluminum chloride solution is added dropwise from a microburet. When the color of the solution becomes a darker yellow as the end point is approached, split drops of the standard solution are added. The solution is boiled very gently and the flask agitated carefully in order not to precipitate the colloidal pink lake which is blue in the precipitated form. At the end point there is a sudden change in color from yellow to pink.

In general, the aliquot of the sample solution to be distilled should be of such size as to require 1 to 2 ml. of the standard aluminum chloride for titration of a 50-ml. aliquot from the first 110 ml. of distillate. The end point is more difficult to detect when larger amounts of fluoride ion are present. This is probably because of interference by the hydrolyzed silica, as no difficulty is encountered in titrating larger amounts of sodium fluoride.

Usually all the fluorine is distilled over in the first two 110-ml. portions of the distillate, about 86% being in the first portion. Better results are obtained for the titration by collecting two portions rather than one 200-ml. portion. Titration of aliquots from the third 110 ml. of distillate gives both a check on the completeness of the separation and a combined indicator and distillation blank. The indicator blank alone is about 0.02 ml. for 50 ml. of solution, while the blank determined on the distillate is of the order of 0.024 ml. and usually remains constant for a particular still as long as the conditions of distillation are carefully standardized.

ANALYTICAL RESULTS

The results obtained for the analysis of 25 organic compounds, carefully prepared analytical samples, are given in Table I. Chlorine and bromine have been successfully determined in several organic compounds containing these halogens both separately and together with fluorine. Decomposition was accomplished with equal success by the methods of both Elving and Ligett (*1*) and Kimball and Tufts (*3*). However, it was found advisable in these cases to use glass ampoules instead of gelatin capsules to contain the samples because of the chlorine content of the gelatin capsules. The benzotrifluoride was obtained from the Hooker Electrochemical Company and the other compounds were prepared by other workers in this laboratory and will be described in future publications. The majority of the compounds were liquids, but some were solids and gases at room temperature. The empirical formulas, the mean value of the fluorine content, as well as the average deviation and the number of determinations for each compound are listed in the table.

SUMMARY

Organic compounds containing fluorine are fused with metallic potassium and the fluoride ion is separated by distillation as hydrofluosilicic acid. The fluoride ion is subsequently titrated with aluminum chloride, using Eriochromcyanine R as an indicator.

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RECEIVED January 25, 1949. The work reported was carried out under contract N6ori-107, T.O. II with the Office of Naval Research. This paper was taken in part from the thesis submitted by Mary Jo Gilbert to the Graduate School of Duke University in partial fulfillment of the requirements for the degree of master of arts, June 1949.

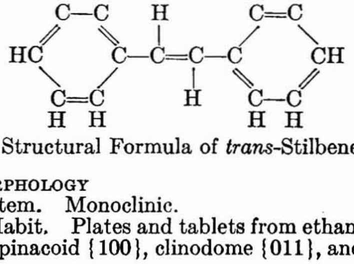
CRYSTALLOGRAPHIC DATA

Contributed by WALTER C. MCCRONE, Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.

26. *trans*-Stilbene

BEGINNING this month an editorial change announced in the November issue becomes effective. Henceforth, the name and sponsoring laboratory for each description will be given full recognition, and full publication credit will be acknowledged to each contributor. With this change it is hoped more data will be submitted than under the past system. As in the past, data should be submitted to W. C. McCrone for completion, checking, standardization, etc.

trans-Stilbene is soluble in most organic solvents and excellent crystals are readily obtained from ethanol, ether, and chloroform. No polymorphism was observed during this study.



Structural Formula of *trans*-Stilbene

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. Plates and tablets from ethanol lying on 100 showing orthopinacoid {100}, clinodome {011}, and clinopinacoid {010}.

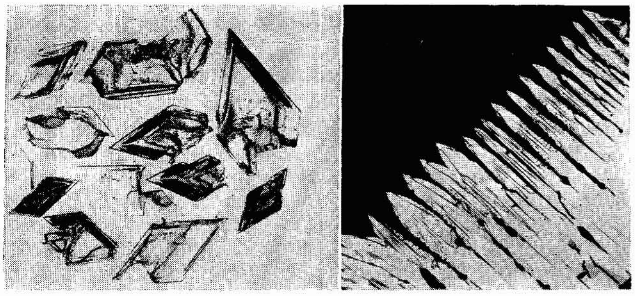


Figure 1. *trans*-Stilbene

Left. Large crystals from ethyl alcohol on slow cooling
Right. Thymol mixed fusion, crossed Nicols

Axial Ratio. $a:b:c$ = 2.77:1:2.17; 2.1702:1:1.4003 (*3*); 1.077:1:1.415 (*1*).

Interfacial Angles (Polar). $011 \wedge 0\bar{1}1 = 126°\ 30'$.

Beta Angle. 114°; 114° 6′(*3*); 113° 48′(*5*); 114° (*4, 6*).

X-RAY DIFFRACTION DATA

Space Group. $P2_1/c$ (*1, 4, 6*).

Cell Dimensions. a = 15.89 A.; b = 5.73 A.; c = 12.42 A. a = 12.35 A.; b = 5.70 A.; c = 15.92 A. (*6*). a = 12.20 A.; b = 5.72 A.; c = 16.00 A. (*5*). a = 12.42 A.; b = 5.73 A.; c = 16.0 A. (*4*). a = 9.6 A.; b = 8.9 A.; c = 12.6 A. (*1*).

Formula Weights per Cell. 4.

Formula Weight. 180.24.

Density. 1.131 (flotation); 1.166 (x-ray); 1.159 (*4*).

Principal Lines

d	I/I_1	d	I/I_1
7.36	0.16	2.77	0.12
5.74	0.31	2.68	0.05
5.37	Very weak	2.60	Very weak
5.17	0.48	2.53	Very weak
4.81	0.40	2.42	Very weak
4.68	0.53	2.35	Very weak
4.55	0.85	2.24	Very weak
4.22	0.20	2.17	Very weak
4.06	0.16	2.11	Very weak
3.90	1.00	2.08	Very weak
3.79	0.40	2.02	Very weak
3.68	0.21	1.94	0.06
3.28	0.25	1.89	Very weak
3.18	0.31	1.85	Very weak
3.10	0.15	1.80	Very weak
2.94	0.05	1.72	Very weak

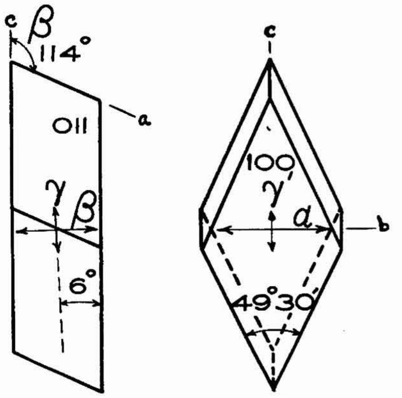


Figure 2. Orthographic Projection of Typical Crystal of *trans*-Stilbene

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.704 \pm 0.002$; $\beta = 1.728 \pm 0.002$; $\gamma = 1.844 \pm 0.005$.

Dispersion Data by Bryant (*2*)

Wave Length	α	β	γ	$2V$,°
3650	(1.797)	(1.975)	(2.03)	53
4047	1.769	(1.864)	(1.973)	90
4358	1.751	(1.823)	(1.940)	80.5
4916	1.726	1.772	1.893	67
5461	1.712	1.743	1.860	57.5
5780	1.706	1.732	1.848	53.5
6234	1.699	1.721	1.835	50
6908	1.691	1.708	1.818	45

Optic Axial Angles (5893 A.; 25° C.). $2V = 52°$. $2E = 96°$; 91° 33′ (*3*).

Dispersion. Axial dispersion with change of sign.

Optic Axial Plane. $\perp 010$ with $\gamma \wedge C = 6°$ in acute β.

Sign of Double Refraction. > 4070 A., positive; < 4070, negative.

Acute Bisectrix. $BX_a \wedge c = 6°$ in acute β ($\lambda < 4070$ A.); $BX_a \parallel b$ ($\lambda \wedge 4070$ A.).

Extinction. $\gamma \wedge c = 6°$ in acute β.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.758$. R (calcd.) = 59.2. R (obsd.) = 65.5.

FUSION DATA

trans-Stilbene melts at 124–125 °C. with some tendency to sublime. The crystals of sublimate are usually rounded, but show the characteristic rhomb shape with a 49°30′ profile angle. The melt supercools somewhat but always solidifies spontaneously to give broad rods growing rapidly parallel to c with a sharp pointed crystal front (profile angle, 49°30′). The optical properties are consistent with the fact that only the view lying on 100 is shown.

It is a pleasure to acknowledge the help of Miss Anne Humphreys in determining the powder x-ray diffraction spacings and intensities.

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Book Reviews

Textile Testing, Physical, Chemical and Microscopic. *John H. Skinkle.* 2nd edition. xii + 353 pages. Chemical Publishing Co., Inc., 26 Court St., Brooklyn, N. Y., 1949. Price, $7.75.

The second edition of this little book has gained through the addition of new material, the revision of old, and a distinct improvement in format. No change has been made in the outline of the book, but the section on statistical methods has been revised and the sections on water resistance and identification of organic finishing agents have been rewritten. The methods for analysis of fiber mixtures and microscopic identification of fibers include the newer commercial fibers. Curiously, the second edition like the first contains no information on the widely used and extremely important tests for color fastness to light, laundering, perspiration, etc., and no explanation is given for the omission of these tests. The revision of the references to literature, listed at the end of each chapter, to include more recent publications is commendable, but the gap between the date of the latest reference, 1945, and the date of publication of the book, 1949, presumably attributable to conditions in the printing industry, is to be deplored. The book is in general clearly and concisely written. It contains a surprising amount of useful information and should be of increased value, especially to students.

WM. D. APPEL

Practical Spectroscopy. *C. Candler.* viii + 190 pages. Hilger and Watts, Ltd., 98 St. Pancras Way, Camden Road, London, N.W. 1, England, 1949. Price, $6.10.

This book was written partly to provide instruction for purchasers of the Hilger wave-length spectrometer and partly as an introduction to general spectroscopy. The first purpose is fulfilled amply; the second purpose leaves much to be desired. The topics treated are qualitative and quantitative spectrochemical analysis, absorption spectra, infrared and Raman spectra, interferometers, and the structure of line spectra. Candler presents the basic principles of each topic in a brief and elementary form, listing some fundamental experiments and a bibliography.

The treatments of the topics and subtopics show considerable variation—for example, Candler describes the measurement of wave lengths rather fully, including even the use of Edser-Butler plates, while his discussions of light sources and sensitometry are much too limited to be of practical or educational value. Much of the theory and application is oversimplified and the bibliographies at the end of each section are incomplete, particularly in so far as developments since 1941 are concerned. The contributions of Sawyer, Duffendack, Owens, Enns, Dieke, Kaiser, and Barnes, to name only a few, are conspicuous by their absence from the text as well as the literature references.

These defects limit the usefulness of the book to the first intended purpose of the publishers as mentioned above. Laboratory analysts and practical spectroscopists will find little new information of practical value in this book.

Jacob Cholak

Physical Methods of Organic Chemistry. *A. Weissberger*, editor. Part I. 2nd ed. ix + 1072 pages. Interscience Publishers, Inc., 215 Fourth Ave., New York 3, N. Y., 1949. Price, $12.50.

This volume is nearly half again as large as the first edition. Three chapters (X-Ray Diffraction, Electron Diffraction, and Refractometry) have been moved forward into a forthcoming Part II and four chapters have been added: Temperature Measurement; Temperature Control (both by J. M. Sturtevant); Determination of Vapor Pressure (G. W. Thomson); and Determinations with the Ultracentrifuge (J. B. Nichols and E. D. Bailey). Ten of the original chapters have been more or less revised: Melting and Freezing Temperatures (E. L. Skau, H. Wakeham); Boiling and Condensation Temperatures (W. Swietoslawski and J. R. Anderson); Density (N. Bauer); Solubility (R. D. Vold and M. J. Vold); Surface and Interfacial Tension (W. D. Harkins) including a section on the Parachor by G. W. Thomson; Properties of Monolayers and Duplex Films (W. D. Harkins); Calorimetry (J. M. Sturtevant); Microscopy (E. E. Jelley); Crystal Form (M. A. Peacock); and Crystallochemical Analysis (J. D. H. Donnay). Three chapters have been rewritten: Viscosity (T. E. McGoury and H. Mark); Osmotic Pressure (R. H. Wagner); and Diffusivity (A. L. Geddes).

The treatment of each chapter varies with the scope of the topic covered; some chapters are more theoretical than others; thus density requires only two or three pages of theory, whereas vapor pressure requires nearly forty for adequate treatment. The authors of the various chapters and the editor must be commended both for achieving their stated purpose and for maintaining throughout what is probably an optimum balance between theory and description.

Any chemist, organic or otherwise, who wants to learn a lot about an instrumental technique or the theory behind it, or who wants to build good equipment, will find this authoritative work invaluable. It is well illustrated and documented, and save for one or two minor duplications—e.g., pages 581 and 648—it is very well edited. The reviewer's copy spends little time on the library shelf.

Thomas R. P. Gibb, Jr.

Manual of Spectroscopy. *Theodore A. Cutting.* 220 pages. Chemical Publishing Co., Inc., 26 Court St., Brooklyn, N. Y., 1949. Price, $6.50.

This manual, the author says, "has been written to assist those who wish to analyze ores, minerals, alloys and inorganic chemicals, or wish to teach others to do so." The contents suggest that any amateur mineralogist can find $10,000 worth of uranium with a $2.50 spectroscope! The history and theory of spectroscopy are presented in 6 scant pages, light sources in 11, spectroscopes in 38, and spectroscopic analysis in 30. The next 85 pages deal with elementary mineralogy and visible spectral lines of 70 elements including such nonspectroscopic material as the discovery, sources, uses, properties, and prices of elements. (The paragraphs on illinium and masurium are obsolete, and the price of xenon is grossly inflated at $600 per liter.) The last 38 pages give approximate wave lengths and intensities of strong lines selected from "M.I.T. Wavelength Tables." A bibliography of 16 items contains 8 works on mineralogy (from 1907), a manual of clinical therapeutics, and the Smyth Report, but ignores all spectroscopic publications since 1939. Obviously, this book will not be of use to anyone except possibly the tyros for whom it was written.

William F. Meggers

Laboratory and Workshop Notes

In the review of "Laboratory and Workshop Notes," by Ruth Lang [Anal. Chem., 21, 1431 (1949)], it was stated that the Institute of Physics is to receive the royalties on the book. In the case of this and other books, the royalties go to the Benevolent Fund of the Institute of Physics, set up to help physicists and their dependents who find themselves in strained circumstances because of some misfortune.

Fifth Southwest Regional Meeting

The Fifth Southwest Regional Meeting, sponsored by the three Oklahoma Sections of the American Chemical Society, was held December 8 to 10 in Oklahoma City. Abstracts of the papers presented before the Section on Analytical Chemistry are given below.

Colorimetric Determinations of Oxidizing Agents Using the Cadmium Iodide–Linear Starch Reagent. Paul Arthur, Thomas E. Moore, and Jack Lambert, Oklahoma A. and M. College, Stillwater, Okla.

A stable starch-iodide reagent, using cadmium iodide and the linear A (butanol-precipitated) starch fraction isolated by Schoch, has been developed for the quantitative determination of oxidizing agents. This reagent is stable indefinitely toward atmospheric oxygen, diffused sunlight, and the growth of microorganisms.

The reagent responds to oxidizing agents according to the pH of the solution. Weak oxidizing agents, such as selenious acid, are reduced only in highly acid solutions, whereas stronger oxidizing agents, such as hypochlorite, react even in neutral solution. The blue starch-iodine color produced shows maximum absorption at about 615 $m\mu$ and obeys Beer's law closely when absorption is plotted against concentration of the oxidizing agent.

Permanent outside standards for the linear starch–iodine blue color can be prepared by adding a small amount of potassium chromate to a solution of nickel hexammine sulfate and making the proper dilutions, always keeping the solutions concentrated with ammonium hydroxide.

A colorimetric method of analysis for trace amounts of selenium (as H_2SeO_3) in water has been developed using the cadmium iodide–linear starch reagent. The reagent is also used in a method being developed for the determination of minute concentrations of chlorine in water in the presence of chloramines.

Biochemical Oxygen Demand of Some Common Organic Compounds Present in Chemical Wastes. E. R. Strong, Jr., C. L.

Shrewsbury, and R. Hatfield, Southwest Research Institute, San Antonio, Tex.

The 5-day B.O.D. values have been determined at 20° C. by the dilution method for some of the common water-soluble organic compounds found in chemical wastes. Original data were obtained for such toxic organics as aldehydes, acids, formates, and alcohols. These data are being used to estimate the pollution characteristics of industrial wastes in treatment and disposal problems.

In this work the B.O.D. was determined for a number of compounds which are normally bactericidal. The B.O.D. values were found to increase with increasing dilution to a point where the toxic effect on the bacteria was nil and then the values become consistent.

An Improved Method for Determination of Sulfur Dioxide in Beer and Foods. Harold E. Weissler, San Antonio Brewing Association, San Antonio, Tex.

The standard method for the determination of sulfur dioxide in beer and food for the past 20 years has been the Monier-Williams method. Despite its inconveniences it has undergone only slight modifications. The method proposed overcomes the objections to the older method without the sacrifice of accuracy.

The entire contents of a cold 12-ounce package of beer are introduced into a carbon dioxide gas-filled system similar to that of Thompson and Toy. Thirty milliliters of concentrated hydrochloric acid are added, the solution is slowly heated to boiling, and refluxing is continued for 1 hour, while a steady, slow flow of carbon dioxide is maintained. The sulfur dioxide expelled from the sample is oxidized by iodine contained in the cold receiving flask. After refluxing is completed the excess iodine is removed by boiling and the solution is neutralized with 0.1 N sodium hydroxide. The sulfate is then titrated with standard barium chloride solution, using tetrahydroxyquinone or potassium rhodizonate as internal indicator in the usual manner.

The results are consistently 0.2 to 0.5 p.p.m. higher than those determined by the Monier-Williams method but are in closer agreement with the gravimetric values than the latter.

Advantages of the method are: Only one standard solution is required and this is easily obtained, prepared, and preserved. The titration is easily performed without the use of electrometric equipment. Some time is saved.

A Field Kit for the Approximate Chemical Analysis of Oil Field Waters. Maryann Duggan and A. V. Metler, Magnolia Petroleum Co., Dallas, Tex.

The construction, equipment, reagents, and method of operating a portable water analysis kit are described. The kit was designed with a view toward economy as well as versatility and simplicity of operation. The pH value of the water is found colorimetrically or by use of a pH meter. Total dissolved solids in the water are found by either density or conductivity measurements. Calcium, magnesium, barium, chloride, sulfate, and sulfide are determined by turbidimetric tests. Turbidimetric standards are furnished in the kit. Ferrous and ferric iron are determined colorimetrically. The method for determining total alkalinity, carbonate, bicarbonate, free carbon dioxide, and hydroxide incorporates the use of two pH measurements and the carbonate equilibrium slide rule of Hirsch. The results obtained with the kit compare well with results obtained using standard water analysis methods for a number of water samples of various types.

Economic evaluation indicates that use of the kit can obviate many of the usual delays and expenses of laboratory analyses. Initial cost of outfitting this kit is low compared to market prices of available water-testing kits.

The tests possible with the kit are adequate for purposes of corrosion control, water repressuring programs in oil fields, identification of oil field brines, boiler water treatment diagnosis, and inhibition of scale formation.

Radiochemical Separation of Tungsten, Rhenium, and Osmium. G. S. Brickam, S. E. Turner, and L. O. Morgan, University of Texas, Austin, Tex.

An ion exchange method for the separation of tracer quantities of rhenium and osmium from larger amounts of tungsten has been developed in order to facilitate the target chemistry of tungsten. In this method, the hydroxide, or free base, form of the anion exchange resin Amberlite IR-4 is used in a chromatographic column. Sample solutions probably contain the elements in the form of tungstate, perrhenate, and perosmate anions at pH 6 to 8. The rate of adsorption of tungstate at this pH is relatively slow, so that if a rapid rate of flow is maintained in the column the tungsten may be recovered quantitatively from the aqueous eluate. Rhenium may then be removed from the column by hydrolysis with ammonium hydroxide. Osmium remains on the column in a reduced form. At high tungstate concentrations, or at a pH lower than 6, appreciable amounts may be retained on the column. In this case, the hydrolytic elution may be controlled, by adjustment of the ammonium hydroxide concentration, to give selective elution of tungsten and rhenium, the tungsten being removed more readily.

Some Errors Involved in the Use of Radioactive Tracers at Very Low Concentrations. L. O. Morgan, University of Texas, Austin, Tex.

The effect of adsorption of ionic or molecular species from solution is often overlooked as a source of error in radiochemical experiments, especially when one or more of the components is present at extremely low concentrations. In experiments in this laboratory by D. Marshall, N. Hackerman, and L. Morgan, otherwise undetectable amounts of sulfur 35 were found to adsorb preferentially on chromium surfaces from active chloride solutions. This gave rise to erroneous interpretations until the situation was recognized. In connection with another problem, the adsorption of perrhenate ions on the glass walls of the transfer system was found to increase to a large extent the apparent width of the elution band of rhenium tracer from a chromatographic column. Others have noted the anomalous behavior of radiocolloids. In experiments with zirconium 95 tracer, considerable difficulty can be encountered unless adequate precautions are taken to minimize the detection of the daughter columbium 95 which is present in very low concentration and which has a marked tendency to colloid or subcolloidal polymer formation in most media.

Determination of Calcium and Magnesium Ions in Water Using the High Frequency Titrimeter. Fred W. Jensen, G. M. Watson, and L. Gutierrez-Vela, Texas A. & M. College, College Station, Tex.

The standard soap method for the determination of total hardness in waters is not too accurate. Instead of utilizing the formation of a permanent foam as an indication of the end point, the use of the high frequency titrimeter was introduced. Soap solutions were standardized by titration with hydrochloric acid in the titrimeter not only more rapidly but with greater accuracy and reproducibility than by the standard method. The interesting form of the titration curve suggests possible uses of the titrimeter in other fields. The determination of calcium by soap solution gave reproducible results which checked the stoichiometric end point better than the standard method. Magnesium was determined by titration with sodium hydroxide in ethanol; again the accuracy was better than by the standard method. Mixtures of calcium and magnesium present difficulties which have not yet been overcome.

A High Frequency Oscillator Utilizing the Heterodyne Principle to Measure Frequency Change Induced by Diverse Chemical Systems. Philip W. West and T. S. Burkhalter, Louisiana State University, Baton Rouge, La.

An instrument which has unusual potential utility in the field of chemical analysis consists essentially of two high frequency oscillators (one a reference and the other a working oscillator) and a detector system for measuring the frequency difference between the two oscillators.

Application of the instrument to chemical analysis 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 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, caused by inserting a chemical system into the tank circuit coil of the working oscillator, is measured by comparing the frequency of the working oscillator with that of the reference oscillator before and after insertion of the chemical system. The magnitude of the frequency change is a function of the composition of the chemical system inserted.

Stability of oscillation, essential for this type of operation, has been checked and found satisfactory. A preliminary investigation of sensitivity and possible application has been made. The instrument has been used to make conductometric titrations, to analyze binary organic mixtures, to detect very small changes in concentration of dilute ionic aqueous systems, and to distinguish between isomers of short-chain saturated alcohols. The following data illustrate the instrument's sensitivity to change in composition: Distilled water caused a frequency change of 4725 cycles, while 0.001 N hydrochloric acid caused a change of 5977 cycles; isopropyl alcohol caused a change of 4025 cycles, while *n*-propyl alcohol caused a change of 4170 cycles. The instrument is sensitive to a frequency change of about 10 cycles.

Determination of Solubilities with the Beckman Du Spectrometer. MADISON L. MARSHALL, Texas State College for Women, Denton, Tex.

Solubility data in the literature are extensive and frequently of a high degree of accuracy. However, the widespread usefulness of such data justifies the extension of solubility studies. Ultraviolet absorption measurements provide a novel method for the determination of the solubility of certain organic compounds in water. Data were supplied for the solubility of benzene and for toluene in water. Comparisons with the literature values were made. Three conditions must be met for applicability of the method: The solute must exhibit absorption in the measurable range, whereas the solvent must be optically clear; the solute must be only slightly soluble; and absorption must obey closely the Beer-Lambert law. Fortunately, the third condition is likely to be met if the second is fulfilled. Possible extension of the method to systems involving aldehydes, ketones, and other classes of organic compounds was proposed.

Quantitative Ultraviolet Analysis of Two Aromatic Hydrocarbon Isomer Systems. JOHN A. PERRY, Monsanto Chemical Co., Texas Division, Texas City, Tex.

Quantitative analytical methods based on ultraviolet absorption spectrophotometry have been set up for the xylenes and ethylbenzene, and for the diethylbenzenes and *sec*-butylbenzene. *n*-Heptane purified by silica gel filtration is used throughout as the solvent. Extinction coefficients are established in a two-step procedure. In the first step, the relative magnitudes of the coefficients are determined merely by introducing an unweighed sample of the pure hydrocarbon into the absorption cell and observing the optical densities at the various selected wave lengths. In the second step, the optical densities observed from synthetics are used to establish the relative magnitudes of the sets of coefficients. This is done by successive solutions of the matrix formed from the coefficients and the observed densities, with indicated adjustments of the sets of coefficients between the solutions.

Apparent deviations from Beer's law seem to be the chief limitation of accuracy and precision of the method. Excellent accuracy (0.2% relative) is available for the middle concentration ranges, and only fair accuracy (5 to 10%) for the extreme concentration ranges. The high accuracy and precision are the result of deriving the values of the constants directly from the experimental conditions; the diminution of accuracy and precision is believed to be the result of uncorrected apparent deviations from Beer's law.

The approach described permits rapid development or revision of multicomponent normalized analyses; the speed is achieved by the elimination of most of the experimental work in the determination of extinction coefficients.

Polarographic Determination of Zinc in Oils. J. T. WILEY, J. E. DELONEY, AND R. L. WINSTEAD, Texas Co., Port Arthur, Tex.

The ever-increasing use of various types of additives by the oil industry has made it desirable to have a rapid and accurate means of controlling the amount of additive present in the oil, and also to assure a homogeneous mixture. This can be controlled by the determination of zinc, where it is one of the metals present in the additive being used.

The polarographic method gives accurate and reproducible results and is much faster than the usual gravimetric or electroplating methods. Zinc has been determined accurately in concentration from 0 to 0.10% by weight, and the method should be applicable to much larger concentrations. Barium, phosphorus, or calcium, other metals which are sometimes present along with the zinc in additives, do not interfere.

The method consists of carefully ashing the oil to remove the organic matter, dissolving the ash in concentrated hydrochloric acid, adding gelatin as a maximum suppressor, making up to a known volume, and determining the polarogram. The percentage of zinc is then calculated from the step height obtained versus known standard samples where zinc oxide is used.

Determination of Molecular Structures in Polyolefinic Hydrocarbon Oils. SIMON MIRON AND R. J. LEE, Pan-American Refining Corp., Texas City, Tex.

The thorough characterization of molecular types present in the complex high-boiling olefinic oils derived by thermal or catalytic treatment of petroleum fractions is not possible by the use of any one scheme present in the literature. It was found possible, by combining the useful features of several existing methods for type analysis of nonolefinic oils and by making various adaptations, to derive a scheme of analysis which is believed to be fairly general for polyolefinic oils. This scheme was illustrated in connection with the analysis of a typical polyolefinic, polycyclic drying resin marketed under the trade name Panapol 2-C.

The oil is selectively hydrogenated in order to saturate only the olefinic linkages, after which it is completely hydrogenated to saturate the residual aromatic bonds. The average number of rings per molecule and the average size of the rings are computed on the completely saturated oil, making use of type formulas and molecular volumes. The true unsaturation is derived from the increase in hydrogen content on selective hydrogenation, whereas the aromaticity is computed from the difference in hydrogen content between the selectively and totally hydrogenated oil. Final results show the percentages of naphthenic, aromatic, and alkyl carbon atoms, as well as the distribution of olefinic double bonds, along the entire boiling range of the oil. The analysis is supplemented by a study of ultraviolet and infrared spectra. In common with any analytical procedure, certain assumptions are necessary. These were pointed out and the limits of uncertainty were defined. The method is also applicable to oils containing conjugated olefinic and/or alkyne linkages.

The Analyst's Calendar

Fifth Annual Microchemical Symposium

THE Fifth Annual Microchemical Symposium, organized under the auspices of the Metropolitan Microchemical Society to stimulate interest in the field of microchemistry and to promote more widespread use of microchemical techniques, will be held in New York, N. Y., February 24 and 25. There is no registration fee, and the public is cordially invited to attend.

At 6:00 P.M. on Friday, February 24, the symposium will open with a dinner at the Hotel Alden, 225 Central Park West. Technical sessions will be at the American Museum of Natural History.

Friday Evening

8:00 Welcome. OLGA R. FRY, Presiding, BELLA LITTMAN, Chairman, Metropolitan Microchemical Society.
Introduction. OLGA R. FRY, Symposium Chairman.
8:15 Rapid Analyses by Means of Emission Spectroscopy. HAROLD HUGHES.
9:15 Microscopy, Textiles, and Dyes. GEORGE L. ROYER.

Saturday Morning

JAMES S. WIBERLEY, Presiding

10:00 Separation and Identification Techniques in Organic Analysis. SIDNEY SIGGIA.
11:00 Identification of Resinous Plastics. T. G. ROCHOW.
12:15 Luncheon, Hotel Alden

Saturday Afternoon

JOHN A. MEANS, Presiding

2:00 Developments in Techniques and Uses of Spot Test Methods of Analysis. PHILIP W. WEST.
3:00 Problems in Agricultural Microchemistry. C. O. WILLITS.

Dinner and luncheon reservations should be made by February 16 with Mrs. Olga R. Fry, E. I. du Pont de Nemours & Company, Inc., Arlington, N. J.

L.S.U. Analytical Symposium

THE third annual Louisiana State University Symposium on Modern Methods of Analytical Chemistry will be held on the campus at Baton Rouge, January 30 to February 2, 1950. Registration will begin Monday morning in the Coates Chemical

Laboratories. Registration fee will be $7, which will include banquet ticket. Fee for students, not including the banquet, is $2.

Monday Morning

A. R. Choppin. Greetings.

Monday Afternoon

Walter J. Murphy. Progress in Analytical Chemistry.
Paul Delahay. Polarography.
Paul Delahay. Amperometric Titrations.

Tuesday Morning

W. C. McCrone. Microscopic Identification of Organic and Inorganic Compounds.
John Flagg. Organic Reagents.
Charles E. White. Fluorometric Methods of Analysis.

Tuesday Afternoon

Norman Wright. Infrared Spectrometry.
Donald Long. Raman Spectrometry.
A. C. Shead. Profile Microscopy.
Social hour and banquet.

Wednesday Morning

W. C. McCrone. Microscopic Identification of Organic and Inorganic Compounds.
John Flagg. Organic Reagents.
Charles E. White. Fluorometric Methods of Analysis.

Wednesday Afternoon

B. L. Clarke. Analytical Chemistry in Quality Control of Product and in Research.
Donald Long. Raman Spectrometry.
G. F. Smith. Perchloric Acid and Its Uses in Analytical Chemistry.
Evening round table discussions.

Thursday Morning

W. C. McCrone. Microscopic Identification of Organic and Inorganic Compounds.
John H. Yoe. Colorimetric Methods of Analysis.
Charles E. White. Fluorometric Methods of Analysis.
Afternoon round table discussions.

Hotel reservations may be made by writing directly to the Hotel Heidelberg, Baton Rouge. Preregistration for the symposium is encouraged and may be made with P. W. West, Coates Chemical Laboratories, Louisiana State University, Baton Rouge 3, La.

Pittsburgh Conference on Analytical Chemistry

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, sponsored by the Analytical Division, Pittsburgh Section, American Chemical Society, and the Spectroscopy Society of Pittsburgh, will be held at the William Penn Hotel, Pittsburgh, Pa., February 15 to 17, 1950. An exposition of modern laboratory equipment will be a part of the conference, and on Thursday afternoon, February 16, Committee E-2 of the American Society for Testing Materials will hold a meeting at which conferees are welcome. The following tentative program has been arranged.

At 8:30 a.m. February 15 registration will begin at the Fort Duquesne Room, William Penn Hotel, and the exposition will open in the Urban Room.

Wednesday Morning

C. Manning Davis, Presiding

10:00 Welcoming Remarks
R. A. Friedel, Chairman, Spectroscopy Society of Pittsburgh
T. C. Bryson, President, Analytical Division, Pittsburgh Section, A.C.S.
Mary E. Warga, University of Pittsburgh
Henry Freiser, Chairman of Exposition
10:30 Philip J. Elving, Pennsylvania State College. Fundamental Operations of Analytical Chemistry
11:15 George R. Harrison, Massachusetts Institute of Technology. Progress in Spectrographic Instruments
12:00 Lunch and exposition recess

Wednesday Afternoon

R. G. Russell, Presiding

2:00 William Seaman, American Cyanamid Co. The Industrial Analytical Chemist in a Changing Scene
2:30 J. Convey, Canadian Bureau of Mines. Spectrochemistry at the Bureau of Mines in Ottawa
3:00 Exposition recess
3:30 Chester M. Alter, Boston University. An Analysis of Analyses
4:15 Van Zandt Williams, Perkin-Elmer Corp. Recent Developments in Infrared Spectrometry

Wednesday Evening

6:30 Conference Dinner, Ball Room. Toastmaster, J. R. Anderson. Main Speaker, H. V. Churchill

Thursday Morning

Session on Analytical Chemistry

Henry Freiser, Presiding

9:00 Harvey Diehl, Iowa State College. Titrations with Ethylenediamine Tetraacetic Acid
9:35 H. H. Willard, University of Michigan. Determination of Fluoride
10:15 Exposition recess
10:40 George A. Perley, Leeds & Northrup. Electrode Problems in Electrometric Measurements
11:35 E. St. Clair Gantz, Gerald C. Whitnack, Clifford J. Holford, and G. B. L. Smith, U. S. Naval Ordnance Test Station, China Lake, Calif. Determination of Nitric Oxide and Nitrogen Dioxide in Admixture
12:00 Lunch and exposition

Thursday Afternoon

Session on Analytical Chemistry

M. L. Moss, Presiding

2:00 C. F. Hiskey, Polytechnic Institute of Brooklyn. Colorimetry Applied to High Absorbance Systems
2:30 H. A. Liebhafsky, General Electric Co. Some Unusual Cases in Photometric Analysis
3:15 Exposition recess
3:35 George Telep and D. F. Boltz, Wayne University. Ultraviolet Spectrophotometric Determination of Molybdenum
3:55 R. E. Kitson, E. I. du Pont de Nemours & Co. Simultaneous Determination of Cobalt, Copper, and Iron
4:25 H. F. Beeghly, Jones and Laughlin Steel Corp. Behavior of Nitrogen and Some of Its Compounds in Steel
4:45 P. E. Wenger, D. Monnier, and Y. Rusani, University of Geneva. Use of Sensitive Reactions in Semiquantitative Determinations (translated and presented by Henry Freiser)

Thursday Morning

Session on Infrared Spectroscopy

J. J. McGovern, Presiding

9:00 N. D. Coggeshall, Gulf Research and Development Corp. Determination of Organic Functionality by Molecular Spectroscopy
9:35 C. W. Young, M. R. Meeks, and V. E. Whittier, Dow Chemical Co. Determination of Low Concentrations

of Low Molecular Weight Polar Solutes in Nonpolar Solvents

10:05 Exposition recess

10:30 John U. White, Perkin-Elmer Corp. Applications of a Double-Beam Infrared Spectrophotometer

11:00 D. D. Friel, E. I. du Pont de Nemours & Co. Analysis of Plant Samples with Infrared Gas Analyzers

11:30 James D. Stroupe, Rohm and Haas Co. Infrared Analysis of Certain C_1-C_4 Hydrocarbon Mixtures

12:00 Lunch and exposition

Session on Emission Spectroscopy

J. R. Churchill, Presiding

9:00 M. F. Hasler, Applied Research Laboratories. Direct-Reading Analysis of Stainless and Tool Steels Covering Wide Ranges of Composition

9:45 R. W. Callon and L. P. Charette, Aluminium Laboratories Limited. Direct-Reading Methods for Analysis of Aluminum and Aluminum Alloys Using Pin Samples

10:20 Exposition recess

10:40 George Wiener, Westinghouse Electric Corp. Effect of Heat Treatment and Composition on Spectrographic Determination of Carbon in Steel

11:15 Galen Porter, Union Carbide and Carbon Research Laboratories. Spectrochemical Determination of Magnesium in Cast Iron

11:40 W. B. McCreary, International Harvester Co. Factors Influencing the Spectrographic Analysis of Silicon in Gray Iron

Thursday Afternoon

Session on Infrared Spectroscopy

F. A. Miller, Presiding

2:00 J. S. Ard and Thomas D. Fontaine, U. S. Department of Agriculture. Triethylamine Addition as a Means of Increasing the Applicability of Infrared-Transmitting Solvents

2:25 H. G. Monteith, Aluminum Research Laboratories. Quantitative Infrared Analysis of Solid Isomeric Mixtures

2:45 J. Seidman, Sinclair Refining Co. Infrared Analysis of *cis*- and *trans*-Decalin

3:10 R. A. Friedel, U. S. Bureau of Mines. Infrared and Ultraviolet Spectra of Phenols

Thursday Evening

G. W. Kelch, Presiding

8:00 Grant T. Wernimont, Eastman Kodak Co. Design and Interpretation of Two-Factor Experiments

This session will be followed by an informal social hour. Admission by badge.

Friday Morning

Session on Ultraviolet Absorption Spectroscopy

R. A. Friedel, Presiding

9:00 Lionel S. Goldring, Roland C. Hawes, Arnold O. Beckman, Charles D. Coryell, and Michael E. Stickney, National Technical Laboratories. Anomalies in Extinction Coefficients at Low Absorbancies

9:25 D. L. Timma, Mound Laboratory. A Modified Beckman Spectrophotometer and Its Performance

9:45 J. R. Anderson and J. J. McGovern, Mellon Institute of Industrial Research. Spectrophotometric Detection and Determination of Thianaphthene in Naphthalene

10:05 Exposition recess

10:30 J. E. Newell, U. S. Rubber Co. Residual Monomer in Polystyrene. A Spectrophotometric Method

11:05 Alvin S. Glessner, Jr., A. C. Whitaker, and N. D. Coggeshall, Gulf Research and Development Corp. Ultraviolet Absorption Analysis for *N*, N^1-Di-*sec*-butyl-*p*-phenylenediamine in Gasolines

11:25 Frank P. Hazlett, Roy B. Hannan, Jr., and Joseph H. Wells, Mellon Institute of Industrial Research. Determination of Anthracene in Crude Anthracene Cakes by Ultraviolet Spectrophotometry

12:00 Lunch and exposition recess

Session on Emission Spectroscopy

Mary E. Warga, Presiding

9:00 B. F. Scribner, National Bureau of Standards. Standardization of Spectrographic Analytical Methods

9:35 J. Sherman, Philadelphia Naval Shipyard. Stability of Spectra as Determinable by Statistical Analysis of Experimental Data

10:00 John Harley, George Peterson, and George Welford, U. S. Atomic Energy Commission. Spectrographic Determination of Beryllium in Air Dust Samples

10:25 Exposition recess

10:45 E. K. Jaycox, Bell Telephone Laboratories. Quantitative Spectrochemical Analysis of Ashes, Deposits, Liquids, and Other Miscellaneous Samples

11:10 J. T. Rozsa, N. A. Grondin, and J. Golland, National Spectrographic Laboratories. Crucible Fusion Technique of Spectrographic Semiquantitative Analysis

11:35 Alfred T. Myers, U. S. Geological Survey. An Improved Cutting Tool for Spectroscopic Electrodes

12:00 Lunch and exposition recess

Friday Afternoon

Session on Emission Spectroscopy

2:00 B. F. Scribner and J. C. Ballinger, National Bureau of Standards. Spectrochemical Analysis of Bronzes by the Porous Electrode Technique

2:25 J. A. Norris, Massachusetts Institute of Technology. Spectrographic Analysis of Beryllium Metal

2:50 Exposition recess

Session on Analytical Chemistry

J. C. Redmond, Presiding

2:00 W. A. Kirklin, Hercules Powder Co. Standardization in Analytical Chemistry

2:20 Calvin Golumbic, Bureau of Mines. Separation and Analysis of Phenols by Countercurrent Distribution

2:55 L. A. Wooten and W. G. Galdner, Bell Telephone Laboratories. Low Pressure Techniques. Determination of Oxygen and Carbon in Metals

3:20 Exposition recess

3:40 E. B. Read and L. P. Zopatti, Massachusetts Institute of Technology. Determination of Oxygen in Zirconium Metal

4:05 L. C. Copeland and F. S. Griffith, New Jersey Zinc Co. of Pa. Manual Polarograph for Rapid Routine Determinations. Lead and Cadmium in Zinc

4:25 Willard P. Tyler and Earl C. Gregg, B. F. Goodrich Research Center. Analytical Polarographic Study of the Bis (dialkylthiocarbamyl) Disulfides and Dialkyldithiocarbamates

4:40 Allen M. Goldstein, Tracerlab Incorporated. Some Uses of Tracer Techniques in Analytical Chemistry

Session on Specialized Techniques

J. H. Lieblich, Presiding

3:10 Harold P. Klug, Mellon Institute of Industrial Research. Recent Applications of X-Ray Diffraction in the Analytical Field

3:45 William Priestley, Jr., Esso Laboratories. Fluorescence Spectroscopy

4:20 Anne L. Conrad and Winifred C. Johnson, Standard Oil Co. of Ohio. Flame Photometer Techniques for Determining Typical Additives in Petroleum Oils

4:45 Richard H. Hughes, Westinghouse Research Laboratories. Microwave Spectroscopy and Chemical Analysis

Third Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 30 to February 2, 1950

Analytical and Microchemical Group, Philadelphia Section, American Chemical Society. Harry Levin, "Microchemistry in the Petroleum Industry," February 7, 1950

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., February 15 to 17, 1950

Fifth Annual Microchemical Symposium. American Museum of Natural History, New York, N. Y., February 24 and 25, 1950

Chemical Institute of Canada, Analytical Division. Royal Connaught Hotel, Hamilton, Ontario, March 2 and 3, 1950. Second annual symposium

Symposium on Molecular Structure and Spectroscopy. Mendenhall Laboratory of Physics, Ohio State University, Columbus, Ohio. June 12 to 17, 1950

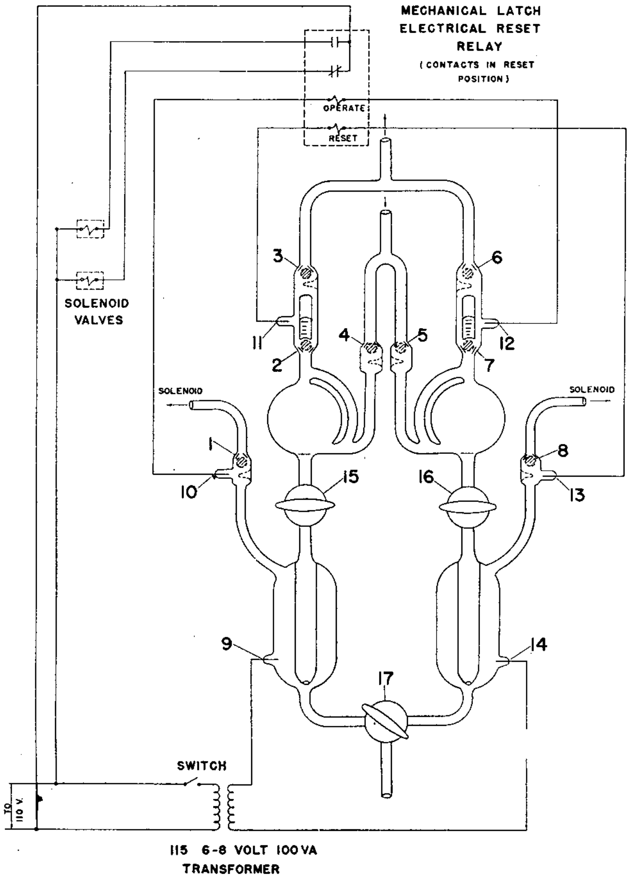
Third Annual Summer Symposium. Ohio State University, Columbus, Ohio, June 16 to 17, 1950

International Microchemical Congress. Graz, Austria, July 2 to 6

AIDS FOR THE ANALYST . . . . .

Glass Circulating Pump. Marvin F. L. Johnson and John A. Glover, Sinclair Refining Company, Harvey, Ill.

Two automatic Toepler pumps connected in parallel may be advantageously employed as a gas circulating pump if means be provided for obtaining proper timing, thus ensuring nearly continuous flow. This type of pump has the advantage of operating by positive displacement of circulating gas, and so may be used to circulate through restrictions such as stopcock bores and at subatmospheric pressures. In the pump described here, continuous flow is obtained by operating the two Toepler pumps one half-cycle apart, and by arranging the electrical circuit and check valves so as to prevent one side from reversing direction until the other side is in a position to do so and to prevent the mercury in either side from proceeding too far in any direction.



Solenoid valves apply vacuum to pump when energized and air pressure when de-energized

Application of compressed air or vacuum through a solenoid valve to the lower reservoirs will raise or lower the mercury in the upper reservoirs, thus forcing gas past check valves 2 or 7 in one case, or drawing in gas past check valves 4 or 5; these check valves are arranged to permit passage of gas only in the indicated directions. (The solenoid valves were obtained from the Automatic Switch Company, Catalog No. 83061, and operate on 110-volt, 60-cycle alternating current, 16 watts. They have $^1/_8$ inch ports, with adapters for $^1/_4$ inch copper tubing on all outlets.) If, therefore, compressed air is being admitted to one reservoir, while the other is simultaneously open to vacuum, one pump will be drawing gas in while the other is pumping out, or vice versa. This is accomplished by arranging the electrical circuit so that contacts 10 and 12 are connected in series with one coil of the relay, contacts 11 and 13 with the other coil, with the voltage applied as shown at contacts 9 and 14. (The double-pole, double-throw relay was obtained from Struthers Dunn, Inc., Catalog No. 51AXA1102; the contacts are designed for 110-volt, 60-cycle alternating current; the coils operate on 6-volt, 60-cycle alternating current.) In this way, reversal of direction takes place only when mercury makes contact at both 10 and 12, or 11 and 13; thus, one coil of the relay is momentarily energized, becoming locked in position, and in turn causes energization of one solenoid, de-energization of the other. When the other set of contacts are made the situation is reversed, and the pumps again change directions.

If the mercury in one side should make contact ahead of that in the other, further travel is prevented by check valves 1, 3, 6, and 8. These valves, and Nos. 4 and 5, consist of conically or spherically ground surfaces, and $^1/_4$ inch steel balls; these balls rest on indentations in the glass, and are floated up by the mercury to the ground surfaces, closing the valve when contact is made. Valves 2 and 7 are similar, but are opened by the balls being floated off the ground surface, and closed when the mercury moves down, leaving an annular ring of mercury to effect a seal. The balls are weighted by mercury-filled glass bulbs, which keep them in place. Stopcock 17 is used for cleaning, filling with mercury, and adjusting the mercury in each pump to the same level.

By proper adjustment of stopcocks 15 and 16 and the sources of vacuum and compressed air, the over-all rate of circulation can be controlled; fine adjustment will lead to better timing of the two pumps and therefore more continuous delivery. The use of 20-cc. bulbs in a system whose total volume is 150 cc. will conveniently circulate 80 cc. of gas per minute, with a total fluctuation in pressure equal to not more than 2% of the total pressure. It is therefore satisfactory for studies of catalytic gas reactions.

Reconditioning of Nernst Glower in Beckman Infrared Spectrophotometer. E. J. Serfass and Mafi M. Chaudhuri, Lehigh University, Bethlehem, Pa.

The infrared spectrophotometer, Model IR-2, manufactured by the National Technical Laboratories, South Pasadena, Calif., utilizes a Nernst glower as a source of infrared radiation. This glower is a small cylindrical tube, 1 to 2 cm. long and about 0.5 mm. in diameter, composed of refractory oxides, which have a negative temperature coefficient of resistance—the property of conducting electricity when hot.

The glower operates from a regulated alternating current supply adjusted to a current consumption of 0.8 ampere. It is heated to a dull red starting temperature by a coil of platinum wire, called the heater, which is mounted close to the glower and can be electrically heated to a bright red. The radiated heat from the heater brings the glower to a starting temperature and the latter starts passing current and also simultaneously operates a relay which turns the heater off.

After continued use, the glower becomes coated with a thin metallic, low-resistance film, which allows the glower to pass current without emitting radiation. Such a glower is considered defective and normally must be replaced by a new one. Frequent replacements are a heavy item of expense, which may, however, be reduced by reconditioning the defective glower by heating with an oxy-gas flame.

The glower, which is brittle, is carefully dismounted from the holder. It may be partially dismounted by removing one end from the holder and carefully bending the platinum leads so as to remove the glower from the heater. A tiny oxy-gas flame is then applied to the glower until the conducting film volatilizes, leaving a white surface. The reconditioned glower is remounted with care and is ready for operation.

The authors have reconditioned a number of glowers in this manner and are using one that has been reconditioned twice.